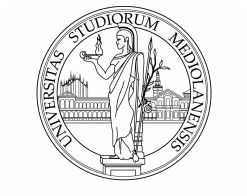

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Sanitary conditions of feral colony cats in the city of Milan

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Index

CHAPTER 1

Foreword

1. **Foreword**

The cat is an animal increasingly widespread; in the Western world, especially in big cities, like Milan, the presence of cat colonies is now an established reality.

The presence of feral cats in the urban environment in Italy has a long history (Natoli, 1994). The size of the feral cat population is unknown, although several sources suggest that it may approximate that of the owned cat population. (Scott et al., 2002). The overpopulation of free-roaming cats is considered to be an important problem because of concerns about animal welfare, wildlife predation, and zoonotic disease transmission (Nutter et al., 2004).

Great part of what is known about the epidemiology of domestic cat diseases, is based on experimental studies carried out in the laboratories, distant from reality. The epidemiology of diseases of cats living under natural conditions in the sociobiological context is a relatively new source of knowledge in the field of epidemiology, especially when an urban stray colony is studied because it may be sentinel for the circulation of new pathogens in the area. The sanitary status of a colony is usually evaluated by a general health examination of the animals and identification of ectoparasites, as well as by additional laboratory tests such as a complete hemogram, platelet count and detection of endoparasites.

Secondly, there is some scarcity of literature on hematologic and urinary reference values in domestic cats (*Felis catus*) in comparison to that published for other species such as rats or Beagle dogs and in particular about how and whether the lifestyle "household" or " feral" may affect some parameters.

The availability of information about general characteristics of the feral cat population, may assist groups in planning programs that make the most effective use of their resources.

The aim of this study is to show a picture general and complete as possible of health status of a feral colony cats in the city of Milan, to determine the

population characteristics, investigate the presence of infectious and parasitic agents, in order to assess impact of feral colony cats on public health, animal welfare, cat overpopulation; and to evaluate the effectiveness of the sterilization program.

1.1 **Feral-colony cats**

Cats have been closely linked to the evolution of humane society for thousands of years, both as real and as symbolic creatures, they have at time been used as scapegoats for natural disasters or personal misfortunes. While some of the negative stereotypes persist to the present days, ever since the 19th century there has been a rapid evolution towards a far more favourable perception of cats.

In many countries the welfare of all cats, and in particular feral cats, has become a focus of public concern. Feral cats are likely to be found wherever humans have travelled, either as escapees from domestication or as deliberately introduced controllers of rodent or other pests. The interest in feral cats may focus on animal control, especially in countries where the free-roaming dog problem no longer is major concern, or on issues such as predation, public health or the well-being of the cats themselves (Slater, 2007). A wide variety of terms are used to describe feral cats, such as free-roaming cats, bars cats, stray cats, etc. This can make difficult the comparisons among the studies. A feral cat can be defined as unconfined, unowned cat regardless of its socialization status. (Levy et al., 2004) Feral cats generally roam freely, usually cannot be handled and is not suitable for placement into a typical pet home. In general, the sociability of a cat relates to its comfort when handled by a person. Some feral cats are completely unfamiliar with humans, some are terrified of them and cannot be handled, some others that have some limited interaction with familiar caretakers are very social and friendly. Some feral or stray cats can be owned cats that are

lost, or have been abandoned by an owner.

A colony is defined as a group of three or more sexually mature animals living and feeding in close proximity (Slater, 2005). Feral cats or colonies can further be described based on "ownership". Some are completely independent of humans and some are provided with food and shelter on a regular basis by "caretakers". A colony is referred to as "menaged" if it is controlled by trap, neuter, and return (TRN) programs (Robertson, 2008). The social structure of a colony may comprise a varying number of cats, they may be cats living alone or great groups (Mendes-De-Almeda et al., 2004).

The main problem concern feral colony cats management is that they are very adaptable and also fecund, giving birth to one to three litters per year of two to six kittens. Since cats are sexually mature at five or six months of age, this can result in a substantial number of cats in a short period, even with high mortality rates (Nutter et al. 2004).

1.1.1 Feral-colony cats in Milan

The current administrative division of Milan, Italy comprises nine "decentralization zones";. Zones are distinguished by a number, from "Zone 1" to "Zone 9".

The population of unowned feral cats in the city of Milan is suspected very large and distributed throughout the whole territory.

In Milan there are more than 450 cat colonies censite (www.comune.milano.it), but it is difficult to estimate the real number of feral cats. They are composed of groups of cats, more or less numerous, spread in all areas of the city and cared for by well-organized volunteers who assist them tirelessly. Protected by national, regional and municipal regulations, the cats now have the status of protected animals in all respects with the right to be cared and treated by the

Veterinary ASL.

Usually the cat colonies that live freely in the territory cannot be moved from the place where they are established. The capture of the cats who live in a state of freedom, as provided by current regulations, is permitted only for sterilization and health care necessary for their well-being.

1.2 **Methods for controlling feral cats populations**

Methods developed for dealing with the population of feral cats can be divided into three main approaches. The first is to kill cats on site, the second is to trap and remove cats for euthanasia or relocation, and the third is to trap, neuter and return cats to the original location. The first method is generally not popular with the public and it is used in location without human habitation such as the islands. For example on Fregate Island (Seychelles), Marion island, Little Barrier Island (New Zeeland). The increasingly public views that cats are domestic animals for whom the man is responsible, and he doesn't accept the killing of cats as a solution to a problem that, in many instances, is due to people introducing cats to the location in the first place.

In the United States, Canada, and Europe, feral cats are most often trapped and removed. After removal usually they are destroyed, most animal control agencies euthanize feral cats that enter their facilities. Some have mandatory holding periods while others determine that the cat is feral on arrival and euthanize it shortly thereafter. A few have programs that place cats with local cat organizations. (Slater, 2005).

In the past decade in North America, there has been an increasing tendency to recommend removal and relocation of cats to another property, often a rural home, farm or sanctuary (Slater, 2005).

The program of trap, neuter and return/release (TNR) program in general,

involves the humane trapping of cats, sterilization by a veterinarian, vaccination for rabies in countries where that is appropriate, permanent identification of sterilization and release back to the original trapping location (Robertson, 2008). The aim of a TNR program is to create a stable population where cats can no longer reproduce; natural attrition will eventually decrease numbers or at least maintain a stable number of cats. Since cats are returned to the original habitat, a vacuum is not left to encourage cats from nearby areas to move in
or remaining intact cats to repopulate. Because there is always the potential for cats to join the colony, the program must continue to trap new cats that migrate into the area. An aggressive adoption program for tame adults and kittens under about eight weeks of age will reduce the numbers of cats in the colony more quickly (Levy et al., 2003). Sterilization decreases roaming of male cats, improves body condition (Scott et al., 2002) and tends to make cats more interactive with their caretakers (Scott & Levy, 2002). Thus, TNR together with adoption and monitoring programs are the most effective and humane options for the long-term control of feral cat colonies. Well studied techniques for anesthesia of feral cats are safe and result in minimal mortality (Williams et al., 2002, Cistola et al., 2004). Newer anesthetic protocols are being developed that focus on postoperative analgesia and a quicker return to normal function (Robertson, 2008)

TNR appears to have originated in South Africa and Denmark well over two decades ago (Slater, 2007). It was then imported into England and from there to the United States, Canada, Europe and many other countries (Remfry, 1996).

In the United States, TNR has become an established approach in some locations and has been on the national and regional agenda of governmental and non-profit organizations since the early 1990's.

German animal welfare organizations usually promote TNR as a control method, with a strong emphasis on the sterilization of cats. TNR program is

practised fairly widely and successfully in Holland, and that its administration has benefited from the long history of animal welfare activities in that country.

In Italy after the promulgation in 1991 of national law No. 281 on the management of pet and the control of feral dogs and cats that introduced the no-kill policy for these species: TNR is the only method permitted for controlling the feral cat population. (Natoli et al., 1994)

The crucial points of Law No.281 (1991) concerning the management of feral cats are:. 1.Feral cats have the right to live free; they are protected and cannot be moved from their colony.

2. Feral cats have to be surgically neutered by the local Veterinary Public Services (VPS) and reintroduced in their colony.

3. Cat care-takers (known also as ‘‘cat-lovers’’) become an institutionalised figure. Colony care-takers are gathered in associations; they can have the official assignment of the management of a cat colony if the local VPS and the office for the animal welfare agree. Management modalities are defined at the local level.

The trap, neuter and return method has become widespread in Italy. More recently, the cats of Rome were given the status of “bio-cultural heritage” .A recent publication describes 10 years of experience with TNR in Rome, Italy (Natoli et al., 2006). These authors reported a general decrease in cat numbers after spay/neuter programs in the city, but their efforts were partly thwarted by the arrival of new cats both by migration into the city and from the abandonment of pet cats within the city. They conclude that a TNR program must be combined with education of pet owners about early sterilization and abandonment of pets.

1.3 **Feral cats welfare**

Concern for the well-being of feral cats should consider not only their health but

also their need for some interaction with humans. Cats in managed colonies appear to be in good health and are able to obtain whatever level of interaction they need with their caretaker, (Slater, 2007) but in particular, as far as cities are concerned the colonies are often located in areas or gardens adjacent to schools, kindergartens, hospitals, increasing the possibility of contact with other cats and immunocompromised subjects, such as sick children. The sanitary control of these animals is of fundamental importance in the protection of public health. Also, often subjects from these colonies are adopted by families who already own other animals and is therefore important to have a clear picture of the distribution of certain pathologies to be able to prevent the spread.

Weight and body condition are good clues to general health in cats. A study of body condition in 105 adult feral cats found they were lean (4 on a scale from 1 to 9) but not emaciated at the time of surgery for neutering (Scott et al., 2002). One year later, 14 cats were revaluated and all of them had a substantial increase in falciform fatpad area and depth and body weight, and an increase of one level in the body condition score. Caretakers judged that their cats were friendlier, less aggressive, less inclined to roam and had improved health and coat condition. Some authors have stated that the mortality rate of feral cats is high and the life expectancy is less than 5 years with causes of death ranging from disease, poisoning, car accidents and attack from other animals which
equates with a poor quality of life. In summary the welfare of feral cats can vary markedly. In some managed colonies it can be good but in other situations it can be extremely poor (Robertson, 2008).

FeLV and FIV viruses are the infectious diseases most frequently studied in cat populations, both because of their impact on cats health and the risk of transmission to other felines. The viruses of family Retroviridae cause persistent infection, in general chronic disease and are directly transmissible. The immunodeficiency virus (FIV) is eliminated in the saliva and is mainly

transmitted by bites and causes an immunodeficiency like disease, vertical transmission is also possible. The higher frequency of this infection in males is ascribed to territorial disputes that always involve much fighting, especially among males (Sellon and Hartmann, 2006). The feline leukemia virus (FeLV) is mainly transmitted by saliva and respiratory secretions and it also induces an immunocompromising syndrome that causes a variety of diseases, including highly malignant neoplasias. Its transmission occur through pacific or non-pacific cohabitation of infected and susceptible cats (Hartmann, 2006) Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are present in both owned and feral cats and can impact adversely on their health. As stated above owned and feral cats may be infected with many other infectious organisms that may concern both cats and humans. Overall, cats in managed colonies have a similar prevalence rate of infection as pet cats (Luria et al., 2004).
Feline coronavirus (FCoV) causes enteric disease but a biotype known as feline infectious peritonitis virus (FIPV) is the aetiological agent of feline infectious peritonitis (FIP), a fatal immune-mediated disease.
Other diseases are occasionally studied in feral cats: parasites such as *Isospora* and *Otodectes* species cause diarrhoea and otitis.

1.4 **Public health and zoonotic disease**

There is a possibility of cats transmitting diseases to humans, but in reviewing the literature there is little information on the actual frequency of zoonotic diseases in which cats can be implicated. Transmission of disease to humans will vary between countries and climatic regions and also depend on the health status of the human population. (Robertson, 2008)
There are many diseases that can be linked to transmission from cats. Many diseases are more likely to be encountered by outdoor cats that can acquire

infections from hunting. Indoor cats are less likely to be sources of human infections. Simple preventive measures, such as washing hands before eating, using gloves when gardening, changing the litter daily, and thoroughly cooking all meat, can reduce the risk of acquiring disease from a cat.

Amongst zoonotic agents transmitted by cats, *Toxoplasma gondii* is the most important parasite of the cats that is transmissible to humans. *Toxoplasma gondii* is an obligate intracellular protozoan that can infect all mammals, who serve as an intermediate host. Cats are the only complete hosts in which the protozoan can undergo both sexual and asexual reproduction. The acute infection is generally self-limiting in immuno-competent humans, but may cause serious disease in immunocompromised humans (AIDS patients in particular) or in foetus during pregnancy (Kravetz and Federmann , 2002). Toxoplasma gondii exists primarily in 3 forms during its life cycle. Oocysts are the product of sexual reproduction and are produced in the small intestine of a cat that has consumed tissue cysts containing T gondii. Oocysts are only produced for 2 weeks in the life of a cat when it first acquires infection, which is usually as a kitten. Oocysts contain infectious sporozoites. Tachyzoites represent the rapidly asexually dividing *T. gondii* within host cells (usually macrophages) before or without an adequate immune response. Bradyzoites are slowly dividing *T gondii* contained by an immune response in tissue cysts. Humans acquire toxoplasmosis predominantly by 1 of 3 mechanisms. The most common mode of acquisition is the ingestion of tissue cysts in undercooked meat. Direct ingestion of oocysts is less common and unlikely to occur from direct contact with a cat. Transplacental transmission of tachyzoites from a mother with primary infection causes fetal infection. Rare cases are attributed to transmission of tachyzoites in blood transfusions. (Kravetz and Federmann , 2002). Disease transmission from cats rarely occurs from direct contact for a number of reasons: first, as stated earlier, cats only release oocysts for approximately 2 weeks of their life, when they first acquire

infection from ingesting tissue cysts. This occurs primarily in kittens that hunt outdoors. Second, once released, oocysts require 1 to 5 days to become infective. Thus, regular cleaning of a cat's litter should diminish contact with infective oocysts significantly. On the other hand, oocysts can survive in soil for years and are resistant to acid, alkali, and detergents. A case-control study of 252 cases of primary toxoplasmosis in pregnant women failed to reveal a significant association with any cat exposure, including having an adult cat or kitten at home, cleaning the litter, or owning a cat that actively hunts. Significant risks were found with consuming undercooked meat and with soil contact without gloves. Soil contact allows oocysts, which are deposited by infected outdoor cats, to ultimately be transmitted by fecal-oral contact. A prospective study in human immunodeficiency virus–infected adults also failed to reveal any association with cat ownership or exposure (Kravetz and Federmann , 2002). The prevalence of *Toxoplasma* infection in feral cats appears to be similar to that in owned cats but the feral cats may be more likely to perpetuate the cat-mouse cycle (DeFeo et al., 2002). The diagnosis of toxoplasmosis is most commonly achieved via serologic testing. Indirect immunofluorescence assays are widely available to detect both IgG and IgM antibodies. IgM antibodies are detectable within a few days of infection, and their levels remain elevated for 2 to 3 months, whereas the levels of IgG antibodies increase 1 to 2 weeks after infection and can remain elevated indefinitely.

Among the gastrointestinal parasites *Toxocara cati* is an important zoonotic agent that may be transmitted by cats. Aberrant migration of the cat roundworm through the viscera of incidental human hosts causes visceral larva migrans. Most human infections are caused by the dog roundworm, *Toxocara canis*, not by the cat roundworm, *Toxocara cati*, possibly because cats are more fastidious than dogs in covering up their feces, thereby limiting exposure. A dog or cat with Toxocara worms in its intestine releases eggs with its feces into sand or soil. The

eggs then require a minimum of 2 weeks under ideal moisture and temperature conditions to develop into infective eggs. Direct contact with an infected animal is an unlikely source of infection. Through fecal- oral transmission after contact with contaminated sand or soil, infective eggs are ingested. In the human stomach, the eggs hatch and release infective larvae. The larvae then migrate through the wall of the small intestine and into the bloodstream. Instead, larvae wander aimlessly and can invade any organ, including the liver, muscle, lungs, retina, central nervous system, and skin.

Cats are known to harbor numerous intestinal microbes that can cause human enteritis. *Giardia* is carried by 2% to 3% of cats; most cases are seen in cats with diarrhea. (Hill et al., 2000) No definitive relationship has been established between feline and human cases, although strong evidence from genetic analysis points to a likely zoonotic association (Kravetz and Federmann , 2002). Additionally, cats may carry dermatophyte fungi, mostly *Microsporum canis* in their hair coat (Kravetz and Federmann , 2002). As stray cats live in close proximity to man, dermatophytoses are becoming a serious public health problem in Europe, especially in the Mediterranean region where the incidence of Microsporum canis infection, particularly in children and immunosuppressed people, has been on a steep increase during recent years. Moreover, all attempts made to eliminate the natural source of infection, mainly represented by feral cats, have failed. For its mitigation, integrated medical and veterinary services, better epidemiological surveillance, more strict rules for animal therapy and TNR programmes to control the population size of free-roaming and feral cats are needed. (Duarte et al., 2010). Cats with sporotrichosis skin lesions can transmit the fungus to humans via direct contact. Cat fleas (C felis) or cat mites (Cheyletiella blakei) can cause either infestation or localized dermatitis.

Overall it would appear that feral cats do not have greater impact on transmissible diseases than free-roaming pet cats (Nutter et al., 2004).

Biological samples collected during TNR programs may give advantage to investigate the frequency of this important infections and parasitic diseases of the feral colony

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CHAPTER 2

Description of procedures

2. **Description of procedures**

This work was carried out on a sample of 266 urban feral cats coming from different colonies in the city of Milan presented to Faculty of Veterinary Medicine, Department of Veterinary Clinical Sciences and (DSCV) as part of trap-neutered-release (TNR) programme between November 2008 and April 2011.

The cats were captured by volunteers using bait traps, delivered to DSCV end after a 12 h fasting they were anesthetized. For safety reasons, cats were not removed from traps until they were unconscious.

The induction of sedation was made using a combination of Tiletamine and Zolazepam (Zoletil 100®, Virbac) plus Tramadol Cloridrato (Altadol 0.5%, Formevet), the anesthetic was injected intramuscularly with a 1-ml syringe through the cage in which they were trapped and the dosage used was calculated on the estimated body weight of each animal (12 mg/kg for Tiletamine/Zolazepam and 1 mg kg for Tramadol). After sedation, an IV catheter was applied to each cat and a support fluid therapy was administred. During surgery; anaesthesia was manteined with isofluorane (Isoflo, Esteve, Italy). Cat's eyes were protected by an ophthalmic lubrificant to prevent drying of the cornea.

Before surgery each cat was administered with benzilpenicellina/streptomycin (Tardocillina ®Fort Dodge Animal Health) as broad-spectrum antibiotic coverage at doses of 2ml/Kg and each animal was submitted to a general health evalutation, a clinical examination and a blood, urine and skin samples were taken.

As for surgery male cats were subjected to orchiectomy, female ones to ovariectomy and pregnant females cats to ovariohysterectomy.

At the end of the surgery the tip of the left (males) or right (females) ear was

trimmed to identify the neutered status of the cats, and for their welfare all of animals received an administration of Ivermectin injected subcutaneously at the dosage of 400 μg/Kg.

Cats were hospitalized in individual cages for 4 days where they were monitored, fed twice a day (after 12 hours from anaesthesia) and then released to the location where they were trapped.

2.1 **Data collection**

For each cat data concerning age, gender, origin, individual characteristics, pregnancy status and physical examination findings were recorded together.

- Cats's age: estimated based on dentition, considering the permanent incisors and canine eruption, and size of the cats. The animals were classified as juvenile (age estimated less or equal to 24 weeks), adult (between 1 and 10 years) and senior (over 10 years).
- Gender: males, females, neutered status
- Origin: indicates the localization of the belonging colony of cats reported by the volunteers that have carried out the capture; based on the 9 Milan decentralization zones.
- Individual_characteristics: for each cat it was reported: breed, fur colour, body weight (BW), body condition score (BCS) (1 to 9 scale where the body condition score is divided into 9 levels ranging from 1 –*emaciated*- to 5 –*ideal*- to 9 -*grossly obese*). (Laflamme et al., 1994), height (from the base of the paw to the apex of the scapula), length (from the tip of the nose to the base of the tail) and torax girth.
- Physical examination findings (healthy and unhealthy cats): for each cat it was reported the presence of one or more of the following clinical abnormalities: lynphadenomegaly, pale mucous membranes, gingivitis, upper respiratory tract

infection, ocular infection. Unhealthy cats were defined cats with the presence of one or more of the of the above-mentioned abnormalities.

- Cats were evaluated for general health and presence or absence of dermatological lesion suggestive of dermatophytosis (localized or generalised miliary dermatitis, excessive scale areas, alopecic lesions, crusted lesions, localised areas of erythema, localised or generalised exfoliative dermatitis, localised or generalised seborrhoic dermatitis, chin acne, kerion, pseudomycetomas, perionyxis) (Moriello and DeBoer, 1999).

2.2 **Sampling**

Shortly after anaesthesia:

- from each cat, blood samples were drawn by aseptic procedure from cephalic or jugular vein using 2,5 ml syringes and 22G needles and were placed in ethylene-diamine-tetacetate (EDTA) treated tubes and in serum separator tube .
- from 61 cats urine samples for urinanalysis were obtained by bladder puncture cystocentesis using 5 ml syringes and 22G needles
- from 100 cats swab specimens for cytological examination were obtained by the external ear canal.
- from 149 cats hairs were collected.
-for 139 cats faecal samples produced during hospitalization were collected for parasitological investigations.

2.3 **Statistical analyses**

All statistical evaluation was performed using a statistical softwere package (MedCalc version 9, Mariakerke, Belgium), with significant set at $P \leq 0.05$.

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CHAPTER 3

Seroprevalence of feline immunodeficiency virus, feline leukaemia virus and *Toxoplasma gondii* in stray cat colonies in northern Italy and correlation with clinical and laboratory data

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3. **Seroprevalence of feline immunodeficiency virus, feline leukaemia virus and *Toxoplasma gondii* in stray cat colonies in northern Italy and correlation with clinical and laboratory data.**

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3.1 **Abstract**

Stray cat colonies in urban and rural areas of Lombardy, northern Italy, were surveyed for seroprevalence of FIV antibodies, FeLV antigen and *Toxoplasma gondii* IgG. Of 316 cats tested, 6.7% were positive for FIV and 3.8% were positive FeLV infection; 203 cats were tested for *T. gondii* IgG antibodies and a prevalence of 30.5% was detected. Statistical analysis tested the influence of provenience, age, gender, health status, and laboratory results on seroprevalence and found male gender and adult age were risk factors for FIV infection. FIV infected cats were more likely to have a decreased RBC count than FIV seronegative cats. No predictors were significantly associated with FeLV and *T. gondii* seropositivity. Colony cats in this study posed a limited risk for retrovirus infection to pet cats allowed outdoors, whereas toxoplasmosis exposure was comparable with the worldwide data.

3.2 **Introduction**

Stray cats may serve as reservoirs of infectious agents for domesticated pet cats, such as feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV), which are important causes of morbidity and mortality in cats.[1] Both these

retroviruses have a worldwide distribution. FeLV is transmitted primarily through the sharing of food and drink containers and by behaviour such as social grooming. FIV is transmitted mostly through bite wounds, but can also be transmitted *in utero* or through colostrum and milk.[2,3] FIV and FeLV prevalence varies widely, depending on lifestyle, gender, health conditions, and other variables of the cat populations examined.[4-13]The American Association of Feline Practitioners and the European Advisory Board on Cat Diseases recommends that the retrovirus status of all cats should be known.[1,14,15] Knowledge of the prevalence of FeLV and FIV infection in stray cat colonies is important to define effective prophylactic and management programs for stray feline populations to guarantee the welfare of the cats themselves and to minimize the threat posed to pet cats with outside access.

Stray cat populations may also play an important role in the transmission of several pathogenic agents to humans. Of the zoonotic agents transmitted by cats, *Toxoplasma gondii,* is the most relevant feline parasite. Although transmission of this enteric coccidian parasite can occur following ingestion of undercooked meat, *T. gondii* oocysts pass into the environment from infected cats and are a significant source of infection for humans and other mammals. Cats are the only source of *T. gondii* oocysts in urban environments, and information on the prevalence of exposure in cats is useful for assessing environmental contamination by the protozoan.[16]

In northern Italy, no recently published data exist for FIV, FeLV and *T. gondii* infection in a large population of stray cats from colonies. The objective of our study was to determine seroprevalence of FIV and FeLV infections and T. gondii exposure among stray cat colonies in urban and rural areas in Lombardy, northern Italy. The correlation of seropositivity with laboratory data and parameters such as origin, gender, age, and health status were also examined and compared between infected and uninfected cats.

3.3 Materials and methods

3.3.1 Sampling and data collection

Blood samples and individual animal data were collected from 316 European shorthair stray cats from urban and rural colonies located in the Lombardy region of northern Italy. These samples were collected by veterinary surgeons at the Faculty of Veterinary Medicine through participation in the trap-neuter-release (TNR) programme between January 2008-January 2010. Cats were trapped by volunteers and delivered to the Department of Clinical Sciences at the University of Milan. Cats were anesthetized with a combination of tiletamine and zolazepam (Zoletil 100,Virbac, Italy) at a dose of 12 mg/kg plus tramadol (Altadol, Formevet, Italy) 1 mg/kg, given intramuscularly in the thigh muscles, based on estimated body weight, while cats were confined in the trap. General anaesthesia was maintained with isoflurane (Isoflo, Esteve, Italy) given by mask. Cats' eyes were protected with an ophthalmic lubricant during anaesthesia to prevent drying of the cornea. At the end of the surgery, one cm of the distal tip of the left ear was removed by placing a haemostat proximal to this position and cutting off the tip with surgical scissors. This permanent mark identified sterilized cats and prevented them from being represented for surgery. Age (estimated based on dentition; animals $\leq$ 6 months of age were considered juvenile, whereas all others were considered adult), gender (male or female) and origin (urban or rural) of each cat were recorded together with data obtained from physical examination of the cats (healthy or unhealthy). Unhealthy cats were defined as cats with the presence of one or more of the following clinical abnormalities: lymph node enlargement, pale mucous membranes, stomatitis, or signs of ocular and respiratory infections. Blood samples were collected aseptically from the jugular vein during anaesthesia and placed in EDTA treated tubes and in serum separator tubes. Cats were hospitalized for five or more days

after the surgery depending on health status and then released to the location where they were trapped. All the procedures were approved by the local authority of the city council.

Within 24h of sample collection, a complete blood cell count was performed on whole blood using an ADVIA 120 System (Siemens Healthcare Diagnostics, Milan, Italy). Cats were categorized in terms of presence or absence of anemia (defined as decreased PCV and/or RBC count and/or Hb), leukopenia, leukocytosis, neutrophilia, neutropenia, lymphocytosis, and lymphopenia. Following separation, sera were tested for antibodies to FIV (relative to gp40 and p24 FIV antigens) and for FeLV p27 antigen with a commercial ELISA kit (Snap FeLV/FIV Combo Plus Test, Idexx Laboratories Hoofddorp, Neederland). Confirmatory tests were not performed as part of this study. *T. gondii* sera IgG antibodies were detected using indirect fluorescent antibody tests (IFAT) performed with a commercial kit (Fuller-laboratories Fullerton, CA USA). Titres ≥1:64 were considered seroreactive and therefore indicative of *T. gondii* exposure.[17]

3.3.2 Statistical analysis

Chi-square analysis and Fisher's exact test were used to test for associations between seropositivity for FIV, FeLV and *T. gondii* and the animal source (urban/rural), gender (male/female), age (juvenile/adult), health status (healthy/unhealthy, presence of clinical abnormalities), CBC abnormalities and co-infection. Any parameters statistically linked to the ELISA seropositivity for FIV and FeLV or to the IFAT seropositivity for *T. gondii* IgG were used in a logistic regression model to test for risk factors associated with the positive ELISA and IFAT test. Analyses were performed using a statistical software package (MedCalc® version 9, Mariakerke, Belgium), with significance set at

P<0.05.

3.4 Results

3.4.1 Sample characterisation

All 316 cats were tested for FIV and FeLV infection, and 203 cats were also tested for *T. gondii* exposure. Results of the sample populations are shown in Table 1.

3.4.2 FIV seropositive cats

The overall FIV seropositivity was 21/316 cats (6.7%). FIV distribution by provenience, age, gender, health status, clinical and CBC abnormalities, and FeLV and *T. gondii* status is presented in Table 2. Factors significantly associated with FIV seropositivity status included age, gender, decreased RBC count and co-infection with FeLV. Logistic regression analysis confirmed significant associations between FIV seropositivity and adulthood (OR 5.9, 95%CI 1.2848 to 27.3637, *P*=0.0225), male gender (OR 5.4471, 95%CI 2.0312 to 14.6074, *P*=0.0008) and decreased RBC count (OR 4.5393; 95%CI 1.6571 to 12.4347; *P*=0.0033).

Mean levels of haematological findings in FIV positive and negative cats are listed in Table 5.

Table 1. Study population characteristics of 316 stray cats from colonies in northern Italy tested for FIV, FeLV infection and *T. gondii* exposure during a trap-neuter-release (TNR) programme. CBC= complete blood count, Hb=haemoglobin, RBC=red blood cell, PCV=packed cell volume

Factor	**Category**	**No and (%)**
Origin of the cats (n=316)	Urban area	226 (71.5%)
	Rural area	90 (28.5%)
Age (n=315)	Juvenile	113 (35.9%)
	Adult	202 (64.1%)
Gender (n=315)	Male	94 (29.8%)
	Female	221 (70.2%)
Health status (n=314)	Healthy	129 (41.1%)
	Unhealthy	185 (58.9%)
Clinical abnormalities in unhealthy cats (n=185)	Lymph node enlargement	130 (70.3%)
	Pale mucous membranes	19 (10.3%)
	Stomatitis	87 (47.0%)
	Signs of respiratory tract infection	30 (16.2%)
	Signs of ocular infection	45 (24.3%)
CBC abnormalities (n=297)	Anaemia	211 (71.0%)
	decreased Hb	48 (16.2%)
	decreased RBC	116 (39.1%)
	decreased PCV	193 (65.0%)
	Leukopenia	38 (12.8%)
	Leukocytosis	11 (3.7%)
	Neutrophilia	20(6.7%)
	Neutropenia	26(8.8%)
	Lymphocytosis	6 (2.0%)
	Lymphopenia	137(46.1%)
FIV test results (n=316)	Positive	21 (6.6%)
	Negative	295 (93.4%)
FeLV test results (n=316)	Positive	12 (3.8%)
	Negative	304 (96.2%)
T. gondii test results (n=203)	Positive	62 (30.5%)
	Negative	141 (69.5%)

Table 2. Tests for a relationship between FIV-seropositive test results in stray cat colonies in northern Italy (21 cats) and risk variables using Fisher's exact test (CI =confidence interval). *P*-values in bold are statistically significant ($P<0.05$). * data from logistic regression analysis CBC= complete blood count; Hb=haemoglobin, RBC=red blood cell, PCV=packed cell volume

Factor	Category	FIV Positive no. (%)	P value of Fisher's exact test	Odds ratio	95% CI
Origin of the cats	Urban area	16 (76.2%)	0.80	1.37	0.49-3.86
	Rural area	5 (23.8%)	0.80	0.73	0.26-2.05
Age	Juvenile	2 (9.5%)	0.01	0.17	0.04-0.76
	Adult	19 (90.5%)	**0.01 (0.02)***	5.76 (5.93)*	1.32-25.21
Gender	Male	13 (61.9%)	**0.002 (0.0008)***	4.27 (5.45)*	1.71-10.69
	Female	8 (38.1%)	0.002	0.23	0.09-0.59
Health status	Healthy	8 (38.1%)	0.82	0.87	0.35-2.18
	Unhealthy	13 (61.9%)	0.82	1.14	0.46-2.84
Clinical abnormalities in unhealthy cats	Lymph node enlargement	10 (47.6%)	0.65	1.31	0.54-3.18
	Pale mucous membranes	2 (9.5%)	0.37	1.71	0.37-7.95
	Stomatitis	3 (14.3%)	0.21	0.41	0.12-1.44
	Signs of respiratory tract infection	2 (9.5%)	1.00	1.00	0.22-4.50
	Signs of ocular infection	5 (23.8%)	0.20	1.98	0.69-5.69
CBC abnormalities	Anaemia	18 (85.7%)	0.14	2.58	0.74-9.00
	decreased Hb	7 (33.3%)	0.06	2.87	1.09-7.53
	decreased RBC	14 (66.7%)	**0.01 (0.003)***	3.41	1.33-8.73
	decreased PCV	16 (76.2%)	0.35	1.79	0.64-5.03
	Leukopenia	1 (4.8%)	0.49	0.32	0.04-2.48
	Leukocytosis	1 (4.8%)	0.56	1.33	0.16-10.92
	Neutrophilia	3 (14.3%)	0.16	2.54	0.68-9.48
	Neutropenia	4 (19.0%)	0.10	2.72	0.84-8.78
	Lymphocytosis	0 (0.0%)	1.00	0.97	0.05-17.76
	Lymphopenia	9 (42.9%)	0.82	0.87	0.35-2.12
FeLV test results	Positive	3 (14.3%)	0.04 (0.07)*	5.30 (4.30)*	1.32-21.28
	Negative	18 (85.7%)	0.04 (0.07)*	0.19	0.05-0.76
T. gondii test results	Positive	5 (38.5%)	0.54	1.46	0.46-4.65
	Negative	8 (61.5%)	0.54	0.69	0.22-2.19

3.4.3 FeLV seropositive cats

The overall FeLV seropositivity was 12/316 cats (3.8%). FeLV distribution by provenience, age, gender, health status, clinical and CBC abnormalities, and co-infection with FIV and *T. gondii* are presented in Table 3. No correlations were detected between FeLV status and gender, age, origin, health status and haematological abnormalities. Co-infection with FIV was the only factor that showed a significant correlation with FeLV positivity (P=0.04) based on Fisher's exact test, but not with logistic regression analysis (OR 4.3046, 95%CI= 0.8470 to 21.8771, P=0.0784).

Mean levels of haematological findings in FeLV positive and negative cats are listed in Table 5.

3.4.4 Toxoplasma gondii IgG seropositive cats

The overall prevalence of *T. gondii* IgG antibodies was 62/203 cats (30.5%). *T. gondii* seropositivity distribution by provenience, age, gender, health status, clinical and CBC abnormalities, and FIV and FeLV status are presented in Table 4. A Chi square test did not detect any correlation between *T. gondii* status and gender, age, origin, health status or haematological abnormalities.

Mean levels of haematological findings in *T. gondii* positive and negative cats are presented in Table 5.

3.4.5 Co-infection

Three of 316 samples (1%) were positive for both FIV and FeLV. Out of 203 samples tested for FeLV, FIV and *T. gondii*, five (2.5%) were positive for FIV and *T. gondii* and three (1.5%) were positive for FeLV and *T. gondii*. No samples were positive to all three agents. The statistical association between FIV and

FeLV detected by the Fisher's exact test was not confirmed by the logistic regression analysis. No statistical association was observed between FIV or FeLV occurrence and *T. gondii* co-infection.

Table 3. Tests for a relationship between FeLV-seropositive test results in stray cat colonies of northern Italy (12 cats) and risk variables using Fisher's exact test (CI =confidence interval).
* data from logistic regression analysis
CBC= complete blood count, Hb=haemoglobin , RBC=red blood cell, PCV=packed cell volume

Factor	**Category**	**FeLV positive no. (%)**	**P value of Fisher's exact test**	**Odds ratio**	**95% CI**
Origin of the cats	Urban area	8 (66.7%)	0.75	0.79	0.23-2.69
	Rural area	4 (33.3%)	0.75	1.27	0.37-4.32
Age	Juvenile	3 (25.0%)	0.55	0.58	0.16-2.21
	Adult	9 (75.0%)	0.55	1.71	0.45-6.45
Gender	Male	4 (33.3%)	0.76	1.18	0.35-4.03
	Female	8 (66.7%)	0.76	0.85	0.25-2.88
Health status	Healthy	3 (25.0%)	0.37	0.47	0.12-1.75
	Unhealthy	9 (75.0%)	0.37	2.15	0.57-8.09
Clinical abnormalities in unhealthy cats	Lymph node	6 (50.0%)	0.56	1.44	0.45-4.55
	Pale mucous	1 (8.3%)	0.53	1.43	0.18-11.73
	Stomatitis	6 (50.0%)	0.10	2.73	0.86-8.70
	Signs of respiratory	1 (8.3%)	1.00	0.86	0.11-6.87
	Signs of ocular infection	3 (25.0%)	0.39	2.06	0.54-7.93
CBC abnormalities	Anaemia	11 (91.7%)	0.19	4.67	0.59-36.78
	decreased Hb	2 (16.7%)	1.00	1.04	0.22-4.90
	decreased RBC	5 (41.7%)	1.00	1.12	0.35-3.62
	decreased PCV	11 (91.7%)	0.06	6.23	0.79-48.91
	Leukopenia	1 (8.3%)	1.00	0.61	0.08-4.86
	Leukocytosis	0 (0%)	1.00	0.95	0.05-17.14
	Neutrophilia	0 (0.0%)	1.00	0.52	0.03-9.07
	Neutropenia	2 (16.7%)	0.28	2.18	0.45-10.50
	Lymphocytosis	0 (0%)	1.00	1.72	0.09-32.27
	Lymphopenia	7 (58.3%)	0.56	1.67	0.52-5.38
FIV test results	Positive	3 (25.0%)	**0.04 (0.07)***	5.30 (4.30)*	1.32-21.28
	Negative	9 (75.0%)	**0.04 (0.07)***	0.19	0.05-0.76
T. gondii test results	Positive	3 (37.5%)	0.70	1.38	0.32-5.98
	Negative	5 (62.5%)	0.70	0.72	0.17-3.12

Table 4. Tests for a relationship between *Toxoplasma gondii* IgG seropositivy in stray cat colonies from northern Italy (62 cats) and risk variables using a Chi square test (CI =confidence interval).
CBC= complete blood count, Hb=haemoglobin, RBC=red blood cell, PCV=packed cell volume

Factor	**Category**	***T.gondii* positive no. (%)**	**P value of Chi square test**	**Odds ratio**	**95% CI**
Origin of the cats	Urban area	31 (50.0%)	0.36	0.72	0.39-1.31
	Rural area	31 (50.0%)	0.36	1.39	0.76-2.53
Age	Juvenile	23 (37.1%)	0.42	0.74	0.40-1.37
	Adult	39 (62.9%)	0.42	1.35	0.73-2.49
Gender	Male	13 (21.0%)	0.34	0.66	0.33-1.35
	Female	49 (79.0%)	0.34	1.51	0.74-3.08
Health status	Healthy	24 (38.7%)	0.83	0.89	0.48-1.64
	Unhealthy	37 (59.7%)	0.83	1.12	0.61-2.07
Clinical abnormalities	Lymph node enlargement	27 (43.6%)	0.95	1.03	0.56-1.88
	Pale mucous membranes	2 (3.2%)	0.77	1.15	0.21-6.47
	Stomatitis	16 (25.8%)	0.92	1.03	0.52-2.04
	Signs of respiratory tract infection	8 (12.9%)	0.20	2.20	0.80-6.00
	Signs of ocular infection	9 (14.5%)	0.90	1.04	0.44-2.43
CBC abnormalities	Anaemia	50 (80.7%)	0.10	2.00	0.95-4.24
	decreased Hb	11 (17.7%)	0.63	1.33	0.59-3.00
	decreased RBC	23 (37.1%)	0.61	1.24	0.66-2.33
	decreased PCV	50 (80.7%)	0.08	2.08	0.98-4.38
	Leukopenia	4 (6.5%)	0.37	0.52	0.17-1.62
	Leukocytosis	2 (3.2%)	0.95	1.48	0.24-9.09
	Neutrophilia	7 (11.5%)	0.20	2.35	0.79-7.03
	Neutropenia	0 (0%)	0.09	0.11	0.01-1.88
	Lymphocytosis	2 (3.3%)	0.18	11.30	0.53-239.07
	Lymphopenia	27 (44.3%)	0.94	1.07	0.58-1.98
FIV test results	Positive	5 (8.1%)	0.74	1.46	0.46-4.65
	Negative	57 (91.9%)	0.74	0.69	0.22-2.19
FeLV test results	Positive	3 (4.8%)	0.96	1.38	0.32-5.98
	Negative	59 (95.2%)	0.96	0.72	0.17-3.12

Table 5. Haematological findings (mean levels ± standard deviation SD) in FIV, FeLV and IgG *T. gondii* seropositive and seronegative cats in a feral cat colony from northern Italy.
(RBC=red blood cell, Hb=haemoglobin, PCV=packed cell volume, WBC=white blood cells)

Param	Ref. range	Unit	Mean patient data ± standard deviation (SD)											
			FIV positive		FIV negative		FeLV positive		FeLV negative		*IgG T. gondii* positive		*IgG T. gondii* negative	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
RBC	6000-10100	x10³/µl	5734	1527	6444	1197	6097	1630	6406	1217	6398	1289	6574	1211
Hb	8.1-14.2	g/dl	8.63	1.81	9.51	1.61	9.04	1.99	9.47	1.63	9.41	1.76	9.69	1.67
PCV	27.7-46.8	%	24.05	5.24	26.29	5.27	24.47	4.86	26.20	5.30	24.45	4.69	25.56	4.47
WBC	6300-19600	/µl	10563	4371	10694	4523	9354	3809	10742	4529	11439	4847	10605	3743
Neutr	3000-13400	/µl	7074.2	3790.4	7241.5	3928.5	6714.5	2933.8	7251.4	3951.3	8402.6	4046.2	7976.6	3327.0
Lymph	2000-72000	/µl	2398.3	1495.2	2500.2	1691.4	1759.9	845.0	2523.9	1676.9	2443.7	1696.0	2038.3	1200.7

3.5 **Discussion**

This study investigated the seroprevalence of FIV, and FeLV infection, and *T. gondii* exposure in stray cat colonies of northern Italy. In Italy, the national Law No. 281 of August 14 1991 on the management of pets and on the control of stray cats introduced a no-kill policy for this species.[18] Thus, TNR programs have been carried out to control stray cat populations in many cities of Italy. TNR programs are considered the most practical, effective and humane way for controlling free-roaming cats because they are intended to decrease reproduction without causing harm to the cats.[19] In Milan, there are more than 450 feline colonies.[20] It is common for volunteer people to care for these stray cats without assuming full ownership of them. This scenario allows for daily direct contact with these cats during feeding as well as indirect contact through fomites, such as clothes and shoes, with their pet cats. People may also contact stray cat colonies at schoolyards and public gardens. Stray cats from colonies are susceptible to infections by viruses of different families, and viruses belonging to the family Retroviridae often cause persistent infections. Controlling transmission of these viruses is difficult because they are transmitted directly, causing both chronic and subclinical infections.[21] Knowledge of FIV and FeLV prevalence in stray cat colonies is important to assess the welfare of the cats themselves, and to assess the threat they pose to pet cats with outside access.

The overall prevalence of FIV infection in our population was 6.7%. Adult age, male gender, and decreased RBC count were significant predictors of FIV seropositivity. The prevalence of FIV infection among adult males was compatible with the knowledge that fighting and biting behaviour of stray cats in colonies is the primary mode of transmission of this infection. FIV is effectively transmitted via bite wounds and higher cat densities would lead to more inter-cat aggression and fighting. Male cats were predisposed to contract FIV infection; as

transmission of FIV is mainly through bite wounds, and male cats, irrespective of their neutering status, are more likely to show territorial aggression and be involved in fights. It is interesting to note that FIV positive status in our study was significantly associated with reduced RBC count even though other red cell indices (PCV and Hb) were not associated. This result could be a consequence of the small number of cats sampled.

No association was evident between FIV infection and health status. FIV can cause an acquired immunodeficiency syndrome that increases the risk of developing opportunist infections, neurological diseases, and tumors.[22] A possible justification for the lack of an association between FIV infection and health status is that FIV in most naturally infected cats does not cause severe clinical signs, and FIV infected cats may live many years without any health problems. In addition, it is possible that some clinical signs, such as those related to neurological symptoms or tumors, would not be evident in the cats of our study due to a lack of historical reference data and because the cats were evaluated under general anaesthesia.

The worldwide data on FIV and FeLV prevalence showed a great variation within and between countries. This variability could be due to a number of factors including differences in population densities, level of neutering, age, gender ratios and interaction between cat colonies. The overall prevalence of FIV in our study was lower than other studies conducted in Italy on stray cat colonies; these estimates ranged from 7% of 490 cats in the Veneto region to 35% of 99 cats from northern Italy. [6,7] The only epidemiological data for Milan city estimated a seroprevalence of 13.3%, but only 30 stray colony cats were tested.[23] Our FIV prevalence was, however, similar to seroprevalence in stray cats in Europe ranging from 6.6% of 196 free roaming cats in Finland to 11.3% of 346 urban stray cats in Belgium.[5,8] Finally, our prevalence estimates were higher than the 5.2% of 533 stray cats in North Florida and 2.4% of 585 stray

and feral cats in Maryland (USA).[4,10]

It has been recommended that positive results for the FIV ELISA be confirmed with a western blot assay.[21] Reported sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the assay used in our study to detect FIV antibodies was 100%, 99.6%, 94.5% and 100%, respectively. [24] Given this information, if a false positive occurred in our study it should be limited since the specificity and PPV of the assay used were nearly 100%. As the ELISA test used in this study for assessment of the antibodies to FIV had a sensitivity of 100%, no false negative results to FIV infection should be expected.

Nevertheless, factors that might lead to a bias could include seropositivity to FIV from maternally derived FIV antibodies and seropositivity following vaccination again FIV infection. The possible presence of maternally derived antibodies to FIV in kittens younger than six months of age are reported to confound interpretation of positive test results.[25] Prevalence of FIV positivity in young cats in our study was low (2/21 cats) and a statistically significant value was found for adult age when compared to positivity in juvenile cats. Therefore, it seems likely that bias of FIV prevalence estimates caused by the possible presence of maternal antibodies was minimal. In addition, administration of the FIV vaccine can result in positive test results through production of antibodies that induce positive ELISA results.[26] The vaccine against FIV has not been licensed and introduced in Italy so no bias for vaccination should be present in our population. In our study, another bias could have affected real seroprevalence for FIV if cats had not yet seroconverted following recent exposure and when FIV-specific antibodies in serum are less than the detection limit of the test.

In contrast to FIV infection, in which prevalence varies significantly, the FeLV infection rate of free-roaming cats is similar throughout the world, ranging from 1% to 8%, and our results support these data. The overall prevalence of FeLV in

our study was slightly higher than the 2.3% of 136 stray cats in Piedmont, Liguria and Val d'Aosta.[3,27] In the only study that tested 30 colony cats from Milan, none were detected as positive for FeLV.[28] In Europe, prevalence of FeLV in stray cats ranged from 1% of 196 free roaming cats in Finland to 7.1% of 198 stray cats in Lisbon.[5,12] In the USA, FeLV seropositivity was detected in 4.3% of 1876 free roaming cats from North Carolina and Florida.[9] In our study, cats with retrovirus infection came predominantly from urban areas. Although it was not statistically significant, such a result may arise because urban animals lead more colonial lifestyles, whereas cats living in rural areas probably do not have as much intraspecific contact, thereby limiting opportunities for horizontal disease transmission characteristic of retroviruses infections. FeLV is more pathogenic than FIV, and was long considered to be responsible for more clinical syndromes (mainly anemia) and subsequent secondary infectious diseases caused by suppressive effects of the virus on bone marrow and the immune system.[22] The lack of association between health status and FeLV infection in our survey could be related to the small number of cats that were FeLV positive and, as for FIV, due to a lack of data about health history and because the cats were evaluated under general anaesthesia.

Reported values for sensitivity, specificity, PPV, and NPV of the FeLV test were 92.3%, 97.3%, 73.5% and 99.4%, respectively.[24] Positive results for the FeLV ELISA should be confirmed with an immunofluorescence antibody test. [29] Because positive assay results were not confirmed by means of an alternative assay in the present study, it is possible that some positive assay results were falsely positive for infection. False negative results were also possible because the sensitivity of the test used was not 100%. Negative assay results are also possible and occur when the concentration of FeLV p27 antigen in serum is less than the detection limit of the test or during the pre-antigenemic stage of infection. A bias that could have lowered the real prevalence of natural FeLV infection in our

population is the possibility that colony cats had received prior vaccination against FeLV. In addition to costs associated with vaccinating feral cats, other considerations on the utility of vaccination of colony cats include uncertainty about what proportion of free-roaming cats are naïve to infectious diseases and would benefit from vaccination; whether administration of a single vaccine is beneficial; and whether cats can mount an adequate immune response when they are stressed by capture, transportation, anaesthesia, and surgery.[30] For these reasons FeLV vaccine, even if available in Italy, was not used routinely in stray colony cats in Lombardy, so no bias regarding the vaccination against FeLV infection could have affected our population. Finally, our FeLV infection prevalence results could potentially be biased if vaccinated domestic cats became integrated into the feral population.

The overall prevalence for *T. gondii* IgG antibodies in our study was 30.5%. This seroprevalence was in agreement with those reported in stray populations of cats from other authors in Italy with prevalence higher than 30%.[7,11, 31] Compared with seroprevalence in stray cats in Europe, our data is intermediate between the lowest prevalence of 24.2% found in Lisbon and the highest (70.2%) found in Belgium.[8,12] Our prevalence is similar to 31.6% of sick domestic cats in a large feline population in USA.[32] Although previous studies have recognized an association between infections with FIV and *T. gondii* exposure, no association was observed in this study as nearly all *T. gondii* IgG positive cats were negative for FIV and FeLV infection.[4] In our study, concurrent retroviral infections were not found to be associated with increased risk for developing *T. gondii* antibodies. Since seropositive cats have likely already shed *T. gondii* oocysts, and these oocysts can remain viable in the environment for many months, serological surveys of anti-*T. gondii* antibodies in these animals could be helpful to identify the proportion of cats that have a role in environmental contamination with faecal oocysts.[17] The high prevalence of toxoplasmosis exposure in a large stray

cat population may seriously contaminate the urban environment. The accidental ingestion of oocysts by contact with soil was found to be among the main factors associated with the infection of pregnant women in European cities, with between 6 to 17% of infections in different cities attributed to soil contact; women from Milan city also participated in this study.[33]

In conclusion, information gathered from FeLV and FIV testing in stray colony cats in northern Italy showed that colony cats do not appear to pose a great risk for retrovirus infection to pet cats allowed outdoors. This low prevalence could be due to a history of management of this population through neutering procedures that preserve the social structure of the cat population and decrease competition for food, shelter, and territory. On the contrary, toxoplasmosis prevalence rates in our study suggested that exposure to T. gondii in stray cats is common and these cats may play an important role in the contamination of the environment with the oocysts. Informing the public about the risk of infection through contact with soil and reducing the populations of stray cats by spaying in TNR programs may represent measures to reduce the risk of human infection. Also, provision of commercial food for stray cat colonies, as practiced by the Milan Municipality for a number of years, could help limit the spread of *T. gondii* into the environment through discouragement of hunting.

Lastly, prevalence rates for both retroviral and *T. gondii* infections may change over time and therefore similar surveys should be repeated to monitor trends. Additional studies and up-to-date data are therefore required for a more complete understanding of feline infections in Europe.

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CHAPTER 4

Survey of dermatophytes and saprophytic fungi in stray cats with and without skin lesions in northern Italy

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4 Survey of dermatophytes and saprophytic fungi in stray cats with and without skin lesions in northern Italy

4.1 **Summary**

Cats are possible reservoirs of zoophilic dermatophytes and for this reason are often blamed as being the main source of human infections, and also in the authors knowledge there are no Italian study on the saprophytic prevalence on the feline coat.

The aim of this study was to determine the prevalence of dermatophytes and saprophytic fungi in stray cats with and without clinical lesions from different colonies in northern Italy and to identify their possible reservoir role.

Stray cats (273) were caught between April 2008 and February 2010 from different colonies of northern Italy near human structures in both rural and urban areas. Their hair was sampled by Mackenzie modified brush technique regardless of the presence or absence of skin lesions attributable to dermatophytosis. All the hair samples were subjected to fungal culture. 15 cats were positive (5.5%). *Microsporum canis* was the most common dermatophyte isolated (13/15). The only other isolated dermatophyte was *Trichophyton mentagrophytes* (2/15). Saprophytic fungi were isolated in 201 cats (73.6%) and the most isolated genera are quite similar to other previous published studies. This study showed a prevalence of dermatophytes in stray cats significantly lower than in other Italian or worldwide studies.

4.2 Introduction

Various fungal organism, as dermatophytes or saprophytes fungi, are frequently

found on the feline hair coat. (Moriello et al., 1991; Moriello et al., 1994; Scott et al., 2000) The most diffuse zoophilic dermatophytes that are primarily animal pathogens belong to the *Microsporum* and *Trichophyton* genera and some species of them may cause dermatophytosis in human, especially children or immunodepressed people (Scott et al., 2000; Chermette et al., 2008).

Cats with dermatophytes on their coat can be clinically affected, subclinically actively infected, or uninfected mechanical asyntomatic carriers (Moriello et al., 1994; Moriello, 2003; Chermette et al, 2008).

The significance of fungal spores on asyntomatic carriers is controversial. The majority of literature reports suggest that such carriage was responsible for human infections and cats are considered to be reservoirs of zoophilic dermatophytes (Al-Doory et al., 1969; Zaror et al., 1987; Lunder and Lunder, 1992; Moriello et al., 1994; Mancianti et al., 2003; Cefarchia et al. 2006). In fact *Microsporum canis* is the main dermathophyte causing *tinea capitis* and *tinea corporis* in human in Italy.Filipello et al., 1996,Romano, 1999)

The prevalence of dermatophytes in cats has been reported with much variability, depending on geographical location, season of sampling, clinical and living conditions(Scott et al., 2000; Moriello, 2003). Some studies report that the dermatophytosis is more frequent in warm humid conditions(Moriello and DeBoer,1991; Sparkers et al. 1991; Moriello et al., 1994; Scott et al., 2000; Moriello, 2003) The prevalence seems also to be greater in cats less than one year old (Moriello et al., 1994; Scott et al., 2000) Also long hair and Persian and Angora breeds seems to be risk factors for feline dermatophytosis, (Moriello, 2003) but not all literature agrees (Boyanowsky et al., 2000). The method used for sampling and diagnosis is also thought to influence the recognition of infected cats with or without clinical sings (Outerbridge, 2006); in cats with generalised lesions or in asymptomatic cats, brushing with McKenzie's technique is recommended (Moriello, 2003).

In asyntomatic or random screening domestic or stray cats the world prevalence of dermatophytes varies from 2% to 88%(Baxter, 1973; Zaror et al., 1987; Moriello and DeBoer, 1991; Sierra et al., 2000; Sparkes et al., 2008). In Italy, dermatophytes were isolated from 13 % to 50% of stray or domestic cats regardless of the presence of symptoms (Filipello et al., 1995; Cefarchia et al., 2004)

In most worldwide and Italian studies *M. canis* is the most isolated dermatophyte (Romano, 1999; Boyanowsky et al., 2000; Natale et al., 2007; Sparkes et al., 2008) *Trichophyton mentagrophytes* is rarely isolated in cats and his prevalence varies from 0.5% to 3% worldwide and also in Italy (Baxter, 1973; Moriello et al., 1994; Romano, 1999; Boyanowsky et al., 2000).

The normal skin microbial flora of cats can include also several saprophytic fungi: commonly isolated are *Alternaria spp., Aspergillus spp., Chrisosporium spp., Mucor spp., Penicillium spp., Rhodotorula spp., and Scopulariopsi spp*.(Moriello and DeBoer,1991; Scott et al., 2000; Khosravi et al., 2003). While saprophytic fungal organism are clinically or less concern in healthy host, immunocompromised cats may develop disease secondary to their proliferation (Boyanowsky et al., 2000) and for this reason it would be important to know their prevalence. In the authors knowledge there are no previous italian study on the saprophytic prevalence on the feline coat.

The aim of this work was to obtain more information about the prevalence of dermatophytes in stray cats in northern Italy, area not previously checked, to evaluate the role of reservoir of these cats for both people and domestic animal that may come in contact with them. The prevalence of saprophytic fungi was also assess.

4.3 Materials and methods

Hair samples were collected from 273 stray cats with and without clinical sign living in north Italy. The cats were caught by volunteers between April 2008 and February 2010 during a trap-neuter-release (TNR) program conducted by the Department of Veterinary Clinical Sciences of the Veterinary Medicine University of Milan. The colonies were located in areas adjacent to human structures such as schools, hospitals, farms and homes, both in rural or urban areas.

Age, gender, breed, hair coat length and habitat (urban or rural areas) were recorded for all cats. Age was estimated by dentition and animals were classified as young (< 1 year of age) and adult (> 1 years of age) (Boyanowsky et al., 2000). Cats were evaluated for general health and presence or absence of dermatological lesions suggestive of dermatophytosis (Moriello, 2003;Chermette et al, 2008).

To evaluate seasonal trends of dermatophytes, the samples of each groups were categorized according to the sampling period into warm season samples (from April to September) and cold season samples (from October to March). Each cat was examined with a wood's lamp for several minutes prior to sample collection. Hair or scales exhibiting typical fluorescence was removed with a pair of sterile haemostats and cultured. Then the hair coat of each cat was brushed with a sterile toothbrush using modified Mackenzie collection method (Moriello and DeBoer,1991; Moriello, 2003).

The hair samples were subjected to fungal culture: all the samples were inoculated by gently imprinting the toothbrush onto the surface of 9 cm Petri dishes containing Sabouraud dextrose agar (added with chloramphenicol 0.5% and actidione 0.4%) on one half and DTM (Dermatophytes Test Medium) agar on the second half. The Petri dishes were incubated upside down in an oven

(MICRA, I.S.Co. S.R.L.) in the dark at a constant temperature of 25 °C and examined daily for three weeks. After three weeks the colonies in the medium were macroscopically and microscopically examined (Moriello, 2003) and identified to species level as dermatophytes. Macroscopic and microscopic examinations were also used to identify saprophytic fungi and yeasts but only to genus level.

Epidemiological data from cats were analysed by Chi-square test, or Fisher's exact test if the expected value was less than 5 in any cell, in order to identified significant differences between the observed dermatophytes prevalence according to age, gender, breed, hair coat length, habitat and season. The prevalence of saprophytic fungi was also calculated for the different isolated genera. The software used was MedCalc® (Version 11.6.1.0, Mariakerke, Belgium) and P value < 0.05 was considered significant.

4.4 **Results**

In our study, fungal isolates were found in 78.4% (214/273) of the cats; 6.1% (13/214) had only dermatophytic growth, 93.0 % (199/214) had only saprophytic growth, while 0.9% (2/214) had dermatophytic and saprophytic growth.

The prevalence of dermatophytes on the hair coat of stray cats of north of Italy was 5.5% (15/273). Only two dermatophytes were cultured and both were zoophilic: *Microsporum canis* (86.7% - 13/15) and *T. mentagrophytes* (13.3% - 2/10) (Table 1).

The most commonly isolated saprophytes were: *Aspergillus* spp., *Penicillum* spp., *Alternaria* spp. and *Mucor* spp. (Table 2). These four saprophytes were found in significantly higher numbers ($P<0.05$) than other less commonly isolated saprophytes or yeast. The number of saprophytic fungi isolated from each cat varied from one (104/201) to three (20/201) genera.

Sex, age, hair-coat length, season of sampling and geographical habitat did not show a significant association with dermatophytes prevalence. Also the presence or absence of skin lesions did not show a significant association. No conclusion was possible on the breed because all the cats were european domestic cats. Cats with saprophytic fungi had a lowest prevalence of dermatophytes when evaluated in comparison of cats without saprophytic presence onto the coat. (Table 1)

Table 1. Epidemiological data of the population, prevalence of dermatophytes in relation to the epidemiological data and P value of Fisher's exact test and Odds ratio (OD) for each risk factor considered.

Variable	*Categories*	Number of subject	%	Number (N) and percentage (%) of cats positive for dermatophytes	P value of Chi square test and Odds ratio (OD)
Sex					
	Female	199	72.9	14/199 (7.0%)	P = 0.067 OD = 5.52 (CI=0.71-42.78)
	Male	74	27,1	1/74 (1.4%)	
Age					
	Young	120	44.0	9/120 (67.5%)	P = 0.198 OD = 1.99 (CI=0.69-5.75)
	Adult	153	56.0	6/153 (3.9%)	
Breed					
	European domestic cats	273	100	15/273 (5,5%)	-
Habitat					
	Urban	149	54.6	11/149 (7.4%)	P = 0.133 OD = 2.39 (CI=0.74-7.71)
	Rural	124	45.5	4/124 (3,2%)	
Coat					
	Short	259	94.9	14/259 (5.4%)	P = 0.781 OD =0.74 (CI=0.09-6.09)
	Long	14	5.1	1/14 (7.1%)	
Season					
	Warm	129	47.3	4/129 (3.1%)	P = 0.100 OD =0.39 (CI=0.12-1.25)
	Cold	144	52.7	11/144 (7.6%)	
Skin lesion					
	Yes	63	23.1	3/63 (4.8%)	P = 0.770 OD = 0.83 (CI=0.23-3.02)
	No	210	76.9	12/210 (5.7%)	
Positivity to dermatophytes					
	Yes	15	5.5	-	-
	No	258	94.5	-	-
Positivity to saprophytic fungi					
	Yes	201	73.6	2/201 (1.0%)	P = 0.0001* OD = 0.05 (CI=0.01-0.21)
	No	72	26.4	13/72 (18.1%)	

* p value <0.05

Table 2: saprophytes fungi isolated alone or together on the same subject in the study cat population. The insulation could be pure or a cat could have more of a saprophyte.

Genus	Number of isolation	%
Aspergillus spp.	71	26.0
Penicillum spp.	68	24.9
Alternaria spp.	49	17.9
Mucor spp.	40	14.7
Cladosporium spp.	26	9.5
Rizopus spp.	18	6.6
Scopulariopsis spp.	16	5.9
Malassezia spp.	8	2.9
Candida spp.	4	1.5
Total	300	100

4.5 **Conclusion**

The prevalence of dermatophytes in our study was 5.5%, equal to that found by Boyanowsky (2000) in pacific western costal USA shelter cats, but lower compared with the prevalence of 25.6% found in 1995 by Khosravi in feline colonies of Iran.

When considering only the italian stray cats studies the prevalence of dermatophytes on the coat is much higher comparing with our result; a study conducted in different parts of Siena (central Italy) found a prevalence of 50% in asymptomatic stray cats (Romano, 1999) and a stray cat study conducted in the north east of Italy detected a prevalence of 27% (Natale et al., 2007). Also considering owner cats randomly chosen in the south of Italy (28%)(Mancianti et al., 2003) or shelter cats with and without symptoms in the central Italy (100%) (Iorio et al., 2007) studies, the prevalence in our study was definitely lower. However the very high prevalence found in the latter study (Iorio et al., 2007) could be due to the origin of the cats from the same shelter.

In our study *Microsporum canis* was the more frequent isolated dermatophyte (86,7%) and this is in agreement with other feline studies conducted in Italy and worldwide (Romano, 1999; Boyanowsky et al., 2000; Natale et al., 2007; Sparkes et al., 2008).

The dermatophitic prevalence in this study was no affected significantly by age, gender, hair coat length, habitat or season of sampling. Interesting to note the lower prevalence of dermatophytes in cats which have saprophytic fungi, but, however, the low total prevalence of dermatophytes in our population not allow any conclusion.

There have been no italian study documenting saprophytic growth on the cat coat and only few world studies (Moriello and DeBoer,1991; Boyanowsky et al., 2000; Khosravi et al., 2003). In our study saprophytic fungi were isolated from 201 cats (73.6%); these prevalence were comparable with data reported in literature and also the isolated species were the same as reported in these previous studies. Interestingly, while the prevalence of dermatophytes varies greatly depending on the sample considered or geographical origin, the prevalence of various genera of saprophytic fungi appears to be the same in different parts of the world (Moriello and DeBoer; 1991,Boyanowsky et al., 2000; Khosravi et al., 2003).

In conclusion our estimated prevalence of dermatophytes was much lower than other Italian studies and these results seem to minimize the role of cats as a reservoir in this area. It would be interesting to evaluate the prevalence of zoophilic dermatophytes and dermatophytosis in humans in the same geographical area. The prevalence and the isolated genera of saprophytic fungi were similar to other feline worldwide studies.

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CHAPTER 5

Prevalence of otitis externa evaluated with otic cytology in a population of feral cats.

“Proposed submition on veterinary dermatology”

5. **Prevalence of otitis externa evaluated with otic cytology in a population of feral cats.**

5.1 Introduction

Otitis externa is a common and multifactorial disorder of the ear canal in dogs and cats. The prevalence in dogs is reported between 10-20% (Griffin, 1996;; Radlinsky et al., 2005), while in the few studies conducted on cats the prevalence is much lower and is reported in 2-10% (Pugh et al., 1974; Ginel, 2002; Radlinsky et al., 2005).

The diagnosis of otitis externa is primarily based on clinical signs (head shaking, ear scratching), physical examination (self-trauma, excoriation, acute moist dermatitis near the base of the ear, aural hematomas, malodor, swelling, pain), otoscopic evaluation and ear cytology (Rosser, 2004).

Cytological examination of exudate in the ear canal is a simple, practical and inexpensive diagnostic test that allows us to assess the presence of infection, bacterial colonization or disorders of keratinization. It provides informations on infectious agents present and the type of immune response. A normal ear canal is light pink and smooth, contains minimal exudates and shows at ear cytology normal cornified squamous epithelial cells and a small numbers of normal resident organisms such as *Malassezia pachydermatis* and *Staphylococcus pseudointermedius* (Cole, 2004). The presence of neutrophils with intracellular bacteria in testifies to the presence of infection, while the mere presence of numerous bacteria and cells mixed with wax epithelial exfoliation are indicative of over-growth of bacteria (Rosser, 2004).

The number of microorganism considered normal is often different between authors. Some authors (Nobre et al., 2001; Ginel et al.; 2002 Tater et al., 2003) have compared cytologic specimens from normal and diseased ears and have identified reference ranges for evaluating the significance of organisms (in particular *Malassezia,* bacteria) and cells present on canine and feline otic cytology. In particular smears should be evaluated for number and types of leukocytes and whether they contain phagocytised microorganism (Chickering, 1988). Literature about prevalence of feline otitis externa is scarce mostly when compared with the references in canine medicine. The main causes of feline otitis externa are mites, in particular Otodectes cynotis is isolated in 6.2 – 69.7% of cats (Sotiraki et al, 2001, Mendes-de-Almeida et al, 2010, Akucewich et al, 2002, Nardoni et al, 2005).

Yeast (Malassezia spp) and bacteria (cocci) may be found in a low number in normal ears or become a perpetuating cause of otitis externa (Rosser 2004).

In particular, the yeast Malassezia (M. pachydermatis, M. sympodialis, M. globosa, M. furfur) is isolated in 23-48.4% of cats without otitis externa and in 63.6- 95.1% of cats with otitis externa (Dizotti et al, 2007, Shokri et al, 2010, Nardoni et al, 2005, Cafarchia et al, 2005).

Most studies on the cat take into account one microorganism present at ear level (especially yeast and mites) in a population of owned cats (Sotiraki et al, 2001, Shokri et al, 2009, Nardoni et al, 2005 , Dizotti et al, 2007). To the author knowledge there is no article on the prevalence of otitis externa in feral cats. In the Akucewich article (2005) about the research of ectoparasites in feral cats during summer Akucewich reports Otodectes cynotis in the ears of 37% of feral randomly selected cats .

Even fewer studies have been conducted on the stray cat population (Akucewich et al, 2002, Mendes de Almeida et al, 2010) while completely missing an epidemiological study on stray cats using ear citology.

The aim of our study was to assess the prevalence of otitis externa in a large population of feral cats in the colony of northern Italy based on a semiquantitative evaluation of ear cytology. The study also characterize the otitis externa in relationship with sex, age, habitat, season of sampling and microorganism isolated at the ear cytology. Valuation of auricolar cytology in feral colony cats provides an additional datum about control of sanitary condition of the feline colonies increasing in number and frequently subjected to TNR. Moreover, colony cats may be a potential source of infection for owned cats that have outdoor access.

5.2 **Material and methods**

Swab specimens for cytological examination were obtained by the external ear canal of 187 stray cats living in rural and urban colonies in northern Italy. The cats were caught between April 2008 and February 2010 during a TNR program (T= trap N= neutered R= release) conducted by Department of Veterinary Clinical Sciences of Veterinary Medicine University of Milan in collaboration with the municipalities.

For all cats, independently by the presence of signs of otitis externa, age, gender (including pregnancy), breed, habitat (urban or rural areas) and season of data collection were recorded. Age was estimated by dentition and animals were classified as young if had less than 6 months of age, as adult if had more of 6 months of age.

The seasons were calculated as follows: winter from December to February, spring from March to May, summer from June to August, autumn from September to November (Scott et al, 2002).

For every cat both ears were sampled with the use of a clean non-sterile cotton swab (Angus 2004). The swab was inserted into the lumen of the ear canal and

swabbed against the surface of the vertical ear canal (at the junction of the vertical and horizontal canal). After the cotton swab was rolled onto 2 glass slides evenly distributing a thin layer of material.

One glass slide was kept unstained, suspended with mineral oil and mounted with a cover slip. The other glass slide was heat fixed, stained with modified Wright's stain (Diff-Quick), suspended with mineral oil and mounted with a cover slip (Angus 2004).

The unstained slides were scanned at a very low power (x100) for the research of mites.

Each slide stained with Diff-Quick was evaluated first at low power (x100) to identify the area of interesting, inflammatory cells and keratinocytes and after at higher power (x400) to evaluated the number of Malassezia and bacteria. The mean number of Malassezia and bacteria was obtained evaluating ten different high-power fields (x400) for each slide stained. An higher power field (X600) was used to recognize different kind of bacteria (cocci or rods).

At each slide was given a score from 0 to 3 for each evaluated parameter: mites, bacteria (cocci and rods), yeasts, polymorphonuclear cells (table 1) (Sotiraki et al, 2001, Nobre et al, 2001, Ginel et all 2002, Tater et al 2003).

Individual scores were then summed to give a total score showing separately the left and right ears if different from each other. In our study, the prevalence of otitis was defined as: percentage of cats that had a cytological total score > or = to 2. Cats that have 2 as a total score, but they had obtained this from the sum of 2 cocci and Malassezia borderline values, were considered healthy. Cats with total score greater than or equal to 2 were considered to have otitis externa.

When it was possible (only in the urban colony cats) was done the direct assessment of the external ear canal with otoscopy and ear canal and ear wax alterations were recorded.

In particular we evaluated alterations of quantity and / or color of ear wax or ear canal alterations (erythema, stenosis, pus, ulcers, mites associates with earwax) .

Table 1: criteria for evaluating the significance of organisms and cells present on otic cytology (based on the mean number obtained evaluating ten different high-power fields for each slide stained)

Cytologycal score	0 (Normal)	1 (Gray zone)	2 (Abnormal – otitis externa)	3 (Abnormal – severe otitis externa)
Malassezia (n°)	≤ 2	> 2 < 12	≥ 12 <25	≥ 25
Cocci (n°)	< 4	>4 <15	≥ 15 <25	≥ 25
Rods (n°)	0	-	≥1 ?	>10
Mites (n°)	0	-	≥ 1	-
Polymorphonuclear cells	0	-	-	≥1

5.3 **Statistical Analysis**

Descriptive statistics were evaluated for the signalment variables (breed, sex, age, habitat), season of sampling. In our population we analyzed the prevalence of otitis externa associated with the presence of mites, Malassezia, bacteria with or without polymorphonuclear cells.

Chi-square test was used to evaluate risk factors (sex, pregnancy, age, habitat, season of sampling) associated with otitis externa. Was also carried out the odds ratios of associations previously evaluated as statistically significant at chi-squared test. The correlation between ear cytologic score and direct otoscopic vision was calculated using the kappa test.

Statistical significance was defined as $P < 0.05$. The data were analysed using commercial software (MedCalc ® (Mariakerke, Belgium).

5.4 **Results**

The composition of our population is shown in table 2.

The survey was carried out on 187 stray cats and all the cats were subjected to microscopical evaluation of ear canal cytology. In our population we had 46/187 male cats (24.6%), 141/187 female cats (75.4%) including 35 pregnant female cats (24.8%).

107/187 cats (57.2%) were adult, 80/187 cats (42.8%) were young. All cats were domestic shorthair. 100/187 cats (53.5%) were from urban area, 87/187 cats (46.5%) from rural area.

68/187 cats (36.4%) were caught and sampled in autumn, 42/187 in summer (23.5%), 44/187 in winter (22.5%), 35/187 in spring (18.7%).

Table 2: composition of population

Factor	**Category**	**no. (%)**
Origin of the cats	Urban area	100 (53.5)
	Rural area	87 (46.5)
Age	Juvenile	80 (42.8)
	Adult	107 (57.2)
Gender	Male	46 (24.6)
	Female Pregnant females	141 (75.4) 35(24.8)
Season of capture	Autumn	68 (36.4)
	Summer	42 (22.5)
	Winter	44 (23.5)
	Spring	35 (18.7)

The examination of obtained samples revealed the presence of otitis externa (cytological score ≥ 2) in 103/187 cats (55.1%) (Table 3). Bilateral otitis was presented in 99/103 cats (96.1%) and monolateral otitis in 4/103 cats (3.9%). 13/99 cats (13.1%) with bilateral otitis showed different cytologycal score in left and right ear.

Malassezia has been highlighted in the cytology of 73/187 (39%) cats with 52/187 (27.8%) cats with score ≥ 2 for Malassezia (12-25 hpf). Cocci were seen in 107/187 (57.2%) cats with score ≥ 2 found in 74/187 (39.6%) cats. Rods are

been identified in 30/187 (16%)cats (all with score ≥ 2). Mites are been identified in55/187 (24.4%) (all with score ≥ 2).

The cytological evaluation of the samples are shown in table 4.

In 2 cats that did not show cytological abnormalities compatible with otitis externa, there was the incidental finding of Demodex cati in the ear canal.

Table 3: frequency of cytological score found in a population of stray cats

Cytological score	Malassezia	Cocci	Rods	Mites
0	141 (%)	80 (%)	157 (%)	132 (70.6%)
1	21 (%)	33 (%)	-	-
2 (otitis externa)	13 (%)	39 (%)	-	47 (25.1%)
3 (sever otitis esterna	33 (%)	31 (%)	26 (%)	1 (0.5%)
Bilateral different score	5	4	2	7

Table 4. Cytological evaluation of 103 cats with otitis externa (cytological score ≥ 2)

Auricolar parasites	N (%)
Malassezia	6 (5.8)
Cocci	22 (24.1)
Otoacariasis	14 (13.6)
Cocci and Rods	10 (9.7) 5 cats with evidence of polymorphonuclear cells
Malassezia + Cocci	5 (4.8) 1 cat with evidence of polymorphonuclear cells
Malassezia +cocci +mites	14 (13.6) 3 cats with evidence of polymorphonuclear cells
Malassezia + cocci +rods +mites	13 (12.6) 4 cats with evidence of polymorphonuclear cells
Malassezia + mites	10 (9.7)
Cocci + mites	2 (1.9)
Cocci + rods+ mites	2 (1.9)

The chi-square test revealed gender as a risk factor statistically significant (p = 0.0354) for otitis externa, and in particular the male sex seems to be a predisposing factor (OR 2.2535, p = 0.0248).

The chi-square test showed habitat as a significant risk factor (p = 0.0168), especially cats come from an urban area were found to be predisposing factors for otitis externa with Malassezia (p = 0.0116, OR = 2.3273). Also sampling season was showed as a risk factor for otitis externa with Malassezia (p = 0.0020), especially summer season seems to be protective against otitis externa with Malassezia (OR 0.3587, p = 0.0311) while winter season seems to be favoring the establishment of the yeast ear infection (OR 3.6774, p = 0.0004).

Winter season proved to be a risk factor for mite otitis (OR = 2.5963, p = 0.0091).

The chi-square test showed pregnancy as a risk factor for bacterial otitis externa caused by cocci (p = 0.0127, OR 2.7048).

For technical reasons only 86/187 patients, all from colonies located in urban areas, were evaluated by direct otoscopy.

40/86 cats (46.5%) showed no alteration of pinna or ear canal, 46/86 cats (53.5%) showed alterations of quantity and / or colour of ear wax or ear canal alterations (erythema, stenosis, pus, ulcers, mites associates with earwax) .

The chi-square test showed a statistically significant correlation (p <0.0001) between results of cytology and the changes observed in the direct otoscopy with a OR of 23.29 (p< 0.0001). K statistic between otoscopic observation and citologic results showed a good correlation: 0.651 (95% 0.491-0.811).

5.5 **Discussion**

To the authors' knowledge, our study is the first that analyze the ear cytology in a large population of stray cats.

The prevalence of otitis externa in our study (103/187, 55.1%) is considerably higher than the values reported in the literature (Baxter and Lawler, 1972; Logas, 1994). The high prevalence can be explained by the type of population sampled. In fact, our population is entirely made up of stray cats with health conditions other than house cats and much more exposed to intra-and interspecific contacts with most major risk of transmission of pathogens such Otodectes cynotis .

In most cases the otitis externa was found to be bilateral (96.1%) in agreement with those reported by Pugh in 1974.

Otoacariasis was found in 55/187 (29.4%) cats in our population. This value is similar to that reported in the study conducted by Akucewich in 2002 in a population of 200 feral cat randomly selected for a TNR. Demodex cati was isolated in 2 subjects (only one mite in both cases) presenting no alterations compatible with otitis externa.

This find may be considered a casual finding and not pathological. Malassezia has been isolated in 73/187 (39%) cats but 52/103 (50.5%) cats with otitis externa showed a cytological score suggestive of otitis externa. The high percentage of Malassezia in patients with otitis externa emphasizes the important role of this in the pathogenesis of otitis externa as a perpetuating factor (Angus 2004).

The results obtained on otitis externa with Malassezia are similar to those obtained by other authors (Nardoni et al, 2005, Dizotti et al, 2007, Shokri et al, 2009). In our study the detection of even only a rods was sufficient to diagnose otitis externa also on the basis of what reported by Tater et al (2003) in which study no rods were detected in ears of healthy subjects.

In our study cocci were found instead in 107 subjects including 74 with a cytology score compatible with bacterial otitis externa.

The risk factors' analysis showed that male cats seem to be predisposed to the onset of otitis externa in contrast to that reported in other study about feline and canine otitis (Pugh et al., 1974; Sotiraki et al., 2001; Nardoni et al., 2004; Saridomichelakis et al., 2007).

Urban cats resulted more susceptible to otitis supported by Masassizia. This facts is probably because cats living in urban areas have a higher density than those living in rural area and therefore a greater possibility of direct transmission of yeast (Nardone et al, 2005).

Winter season resulted predisposing factor for otitis by Malassezia and mites. Pregnancy seems to be a risk factor for otitis by cocci. The Chi Square test showed a statistically significant association between direct examination of external ear canal and cytological score. The K statistics showed a good grade of assessment between direct ear canal examination and cytological score. Despite this, the results highlight the need to make both diagnostic procedures because we found cases in which a normal direct observation of ear canal correspond a pathologic cytological score.

5.6 **References**

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CHAPTER 6

Population characteristics of feral colony cats in the city of Milan

6. **Population characteristics of feral colony cats in the city of Milan**

6.1 Introduction

The population of unowned feral cats in the city of Milan is suspected very large and distributed throughout the whole territory. In Milan there are more than 450 cat colonies censite (www.comune.milano.it), but it is difficult to estimate the real number of feral cats. Feral colony cats constitute an important and controversial problem for the impact on public health, cats overpopulation and animal welfare. In Italy TNR program is the only method permitted for controlling the feral cat population. This program allows to obtain very large informations about characteristics of cats populations. The objective of this study was to determine the population characteristics of feral colony cats admitted to TNR program in the city of Milan.

For material and methods see Chapter 2

6.2 **Statistical analysis**

Body weight, BCS, length, girth and height (males versus females and adult versus young) were compared using an unpaired *t* test. Differences were considered statistically significant when $p < 0,05$.

6.3 **Results**(table 1)

All 266 urban feral cats were European, 171 cats (64.5%) were female, 90 (34.0%) were male, 3 (1.1%) were neutered females and 1 cat (0.4%) was neutered male. Gender was not recorded in one cat. At the time of surgery 34

females was in pregnancy status (37.8%).

Cats judged to be young were 118 (44.5%), adult were 145 (54.7%) and 2 cats (0.8%) were judged as senior. Age was not recorded in one cat.

3 cats came from colonies located into zone 1 of Milan (1,1%), 11 cats came from colonies located into zone 2 (4.1%), 113 cats came from colonies located into zone 4 (42.5%), 12 cats came from colonies located into zone 5 (4.5%), 27 cats came from colonies located into zone 6 (10.1%), 55 cats came from colonies located into zone 7 (20.7%), 23 cats came from colonies located into zone 8 (8.6%) and 22 cats came from colonies located into zone 9 (8.3%) (Figure 1).

Among the different coat markings and colours found 63 were silver mackerel tabby (23.7%), followed by 52black with white (19.5%), 32 solid black (12%), 28silver mackerel tabby with white (10.5%), 25 black torties with white (9.4%), 23 silver with white (8.6%), 16 solid blue (6%), 5 red tabby (1.9%), 4 red with white (1.5%) 4 seal point (1.5%), 3 brown mackerel tabby (1.1%), 3 silver classic tabby (1.1%), 3 solid white (1.1%), 2 solid chocolate (0.8%), 1 chocolate with white (0.4%), 1 solid cream (0.4%) and 1 seal tortie point with white (0.4%).

The greater part of cats are short coated 248 (93.2%), 14 cats are semi long coated (5.3%) and 4 are long coated (1.5%).

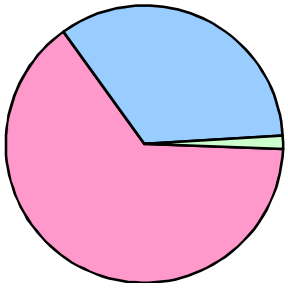
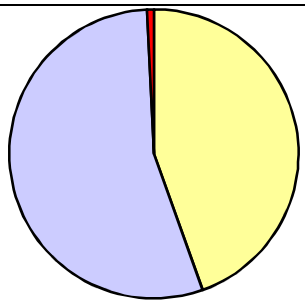
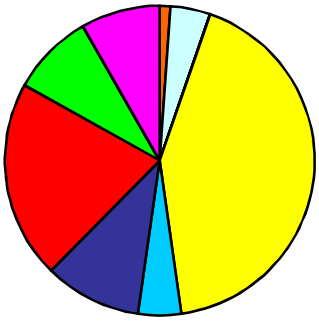
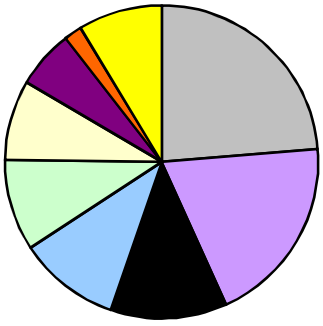
At the time of surgery, the cats were normal/lean and not emaciated (BW 3.1 ± 0.8; BCS 4.3 ± 0.7); the middle length was 51.1 ± 5.9 cm, middle height was 26.3 ± 3.5 cm and middle girth was 29.0 ± 4.4 cm. The following data were not recorded: body weight in 12 cats, body condition score in 18 cats, length in 34 cats, height in 33 cats and girth in 34 cats.

76 cats (28.6%) were found healthy at clinical examination, and 190 (71.4%) resulted unhealthy cats with the most frequent clinical manifestation that was lynfoadenomegaly (n 136/266, 51.1%), followed by gingivitis (n 106/266, 39.8%), ocular infection (n 42/266, 15.8%), upper respiratory tract infection (n 23/266, 8.6%) and pale mucous membrane (n 15/266, 5.6%).

All the data about sample population are showed in the Table 1

The BCS, BW, length, height and girth of cats are summarized in table 2 and table 3

Table 1. Study population of feral colony cats from the city of Milan

Factor	Category	Samples			Graphic
		Number	Proportion	%	
Gender	Male	90	90/265	34.0	
	Female	171	171/265	64.5	
	Pregnancy status	34	34/171	37.8	
	Neutered male	1	1/265	0.4	
	Neutered female	3	3/265	1.1	
Age	Young	118	118/265	44.5	
	Adult	145	145/265	54.7	
	Senior	2	2/265	0.8	
Origin	Zone 1	3	3/266	1.1	
	Zone 2	11	11/266	4.1	
	Zone 3	0	0/266	0	
	Zone 4	113	113/266	42.5	
	Zone 5	12	12/266	4.5	
	Zone 6	27	27/266	10.1	
	Zone 7	55	55/266	20.7	
	Zone 8	23	23/266	8.6	
	Zone 9	22	22/266	8.3	
Coat marking and colour	silver mackerel tabby	63	63/266	23.7	
	black with white	52	52/266	19.5	
	Solid black	32	32/266	12	
	silver mackerel tabby with white	28	28/266	10.5	
	black torties with white	25	25/266	9.4	
	Silver with white	23	23/266	8.6	
	Solid blu	16	16/266	6	
	Red tabby	5	5/266	1.9	

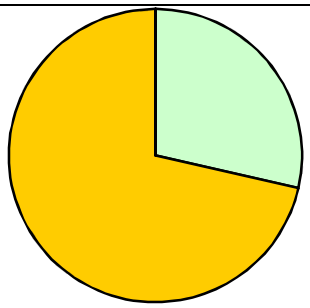
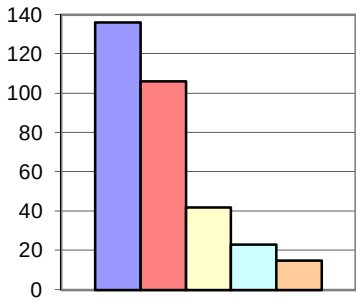
	Red with white	4	4/266	1.5	
	Seal point	4	4/266	1.5	
	Brown mackerel tabby	3	3/266	1.1	
	Silver classic tabby	3	3/266	1.1	
	Solid white	3	3/266	1.1	
	Solid chocolate	2	2/266	0.8	
	Chocolate with white	1	1/266	0.4	
	Solid cream	1	1/266	0.4	
	Seal tortie poit with white	1	1/266	0.4	
Health status	Healthy	76	76/266	28.6	
	Unhealthy	190	190/266	71.4	
Clinical abnormalities	Lynphadenomegaly	136	136/266	51.1	
	Gingivitis	106	106/266	39.8	
	Ocular infectin	42	42/266	15.8	
	Upper respiratory tract infection	23	23/266	8.6	
	Pale mucous membrane	15	15/266	5.6	

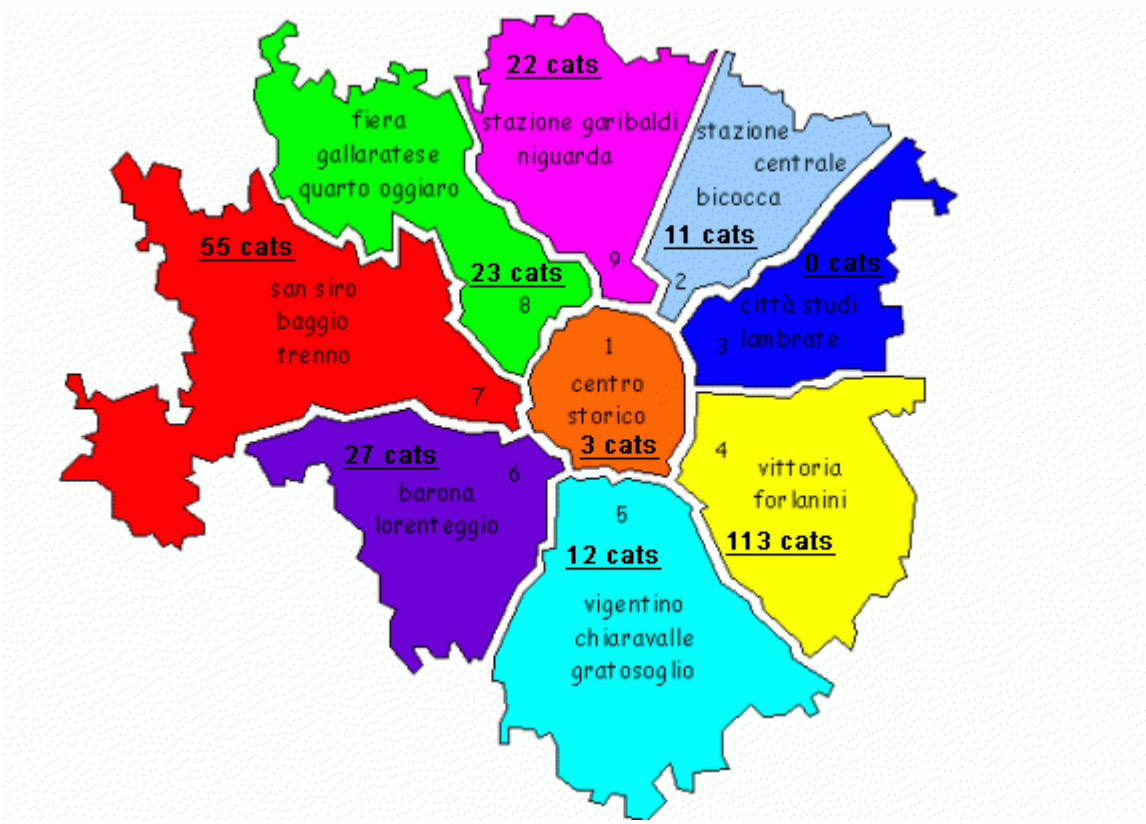
Table 2: BCS, BW of feral colony cats in Milan

BW				BCS		
	n	mean±sd	p	n	mean±sd	p
TOT	255	3.1 ± 0.8		248	4.3 ± 07	
Females	164	3.0 ± 0.7	0.0004	161	4.2 ± 0.7	0.0554
Males	84	3.4 ± 0.9		81	4.4 ± 0.8	
Adult	140	3.5 ± 0.7	< 0.0001	136	4.3 ± 0.8	0.948
Young	112	2.6 ± 0.7		109	4.3 ± 0.6	

Table 3: Length, Height and Girth of feral colony cats in Milan

	LENGTH			HEIGHT			GIRTH		
	n	mean±sd	p	n	mean±sd	p	n	mean±sd	p
TOT	232	51 ± 5.9		233	26.3 ± 3.5		232	29 ± 4.4	
females	148	50.6 ± 5.4	0.111	148	25.6 ± 3.2	0.0002	148	28.6 ± 4.6	0.098
males	79	52 ± 6.9		80	27.4 ± 3.8		79	29.6 ± 3.8	
adult	120	52.7 ± 5,0	<0.0001	120	27.8 ± 2.7	0.0001	120	30.8 ± 4.4	< 0.0001
young	109	48.3 ± 5.7		110	24.5 ± 3.4		109	27 ± 3.4	

Figure 1: Milan decentralization zones.



6.4 **Discussion**

Most of the cats admitted to TNR program were female (64%) and several of them have been presented in pregnancy status (37,8%). This fact reflects the

tendency of Milan caretakers to neuter primarily females cats suspected to be pregnant in order to avoid kittens birth and try to stem the exponential expansion of the colonies.

The fact that 4 cats (3 females and 1 male) already neutered and not marked were captured, shows the importance of auricular tipping after surgery to avoid a useless capture, anaesthesia and surgery (in case of females). The sample made almost entirely of young and adults cats could emphasizes the reduced longevity of feral cats compared to domestic cats, many of which even surpass 10 years threshold. Males were heavier and higher than females as confirmed by the comparison between gender/body weight and gender/height, that was significant. Analysis of the population based on age showed a similar number of kitten (<1 hear)(45.5%) in comparison to adults(54.7%). This composition suggests low infant mortality.

Most of the cats were classified as unhealthy (71.4%), but also for the presence of small clinical abnormalities (mostly lymphadenopathy and gingivitis). In fact most of cats showed an good state of nutrition.

There are several methods to quantify body condition in live animals. Body weight is one method, but it does not differentiate between a lean large cat and an obese small one. Body condition scoring (BCS) (Laflamme et al., 1994) is a method to rank a cat's condition visually. It can be reliable in the hands of an experienced person. Being the body condition scoring a method of judging the overall health and welfare of feral cats (Scott et al., 2002), assessment of BCS in this study shows that the feral cats in Milan are in good conditions of health and appear to be properly managed. Even previous studies found, no significant differences in body weights of free roaming cats compared with pets; and commonly, free-roaming cats were in adequate body condition (Kalz et al., 2000; Scott et al. 2002; Levy et al. 2003).

6.5 **References**

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CHAPTER 7

Hematologic values of feral colony cats in the city of Milan

7. **Hematologic values of feral colony cats in the city of Milan**

7.1 **Materials and methods** (see also Chapter 2)

Within 24h of blood samples collection, a complete blood cell count was performed for 152 samples using an ADVIA 120 System (Siemens Medical solution Diagnostic/Germany). A blood count included determination of red blood cell count (RBC), packed cell volume (PCV), haemoglobin (Hb), total and differentiate white blood cell count (WBC), platelet (PLT) and reticulocyte (RET) count.

7.2 **Statistical analysis**

A descriptive statistic was used to show distribution of hematologic parameters. Data were statistically analized, haematological values were compared using a unpaired *t* test (males versus females, adult versus young, healthy versus unhealthy and pregnant versus non pregnant females). Differences were considered statistically significant when $p < 0,05$.

7.3 **Results**

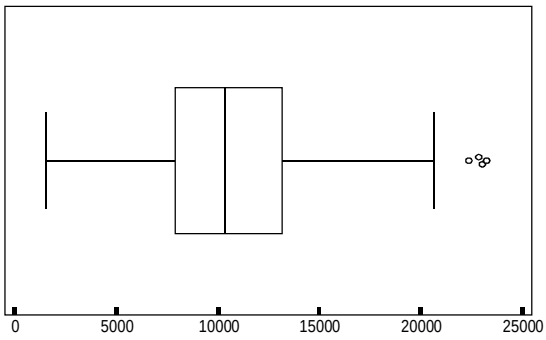
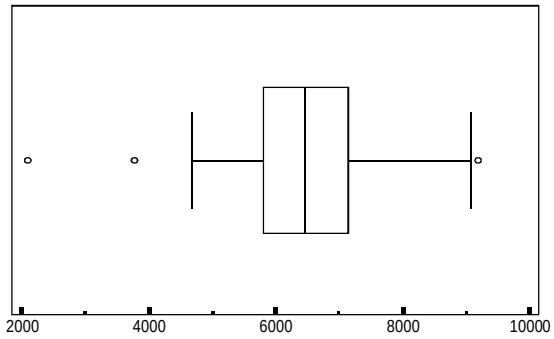
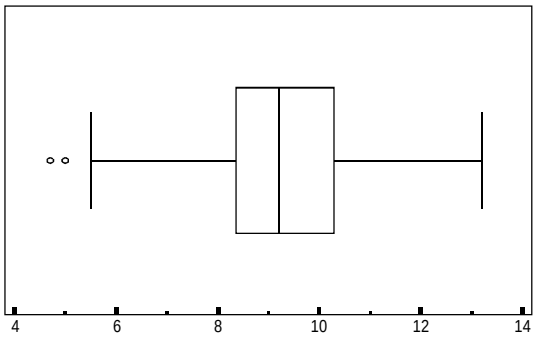
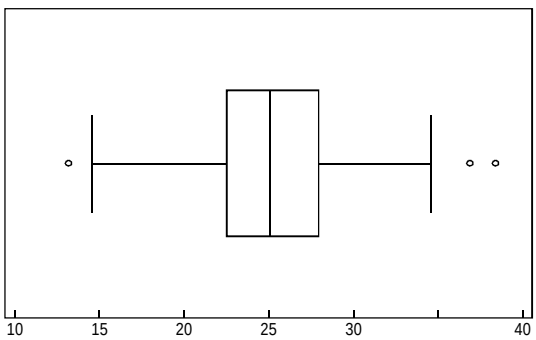
CBC was performed in 152 cats. All data about this sample population are shown in Table 1. Of this 152 cats 106 (69.7%) were female, 41 (27.0%) were male, 3 (2.0%) were neutered females and 1 cat (0.7%) was neutered male. The gender was not recorded in one cat and 18 females were in pregnancy status (17.0%). Cats judged to be young were 70 (46.0%), adult were 81 (53.3%) and 1 cat (0.7%) was judged as senior. Cats were usually in good body condition (94.6% BCS 4-5/9), BCS was not recorded in 5 of the above cats. 46 cats

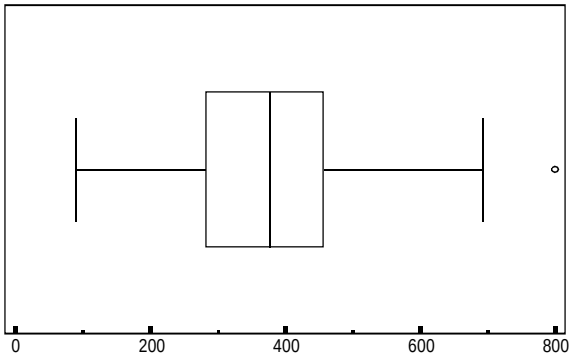
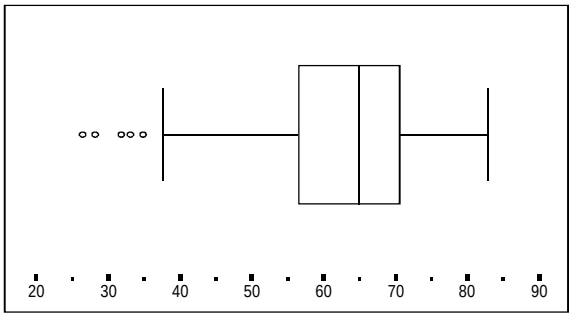
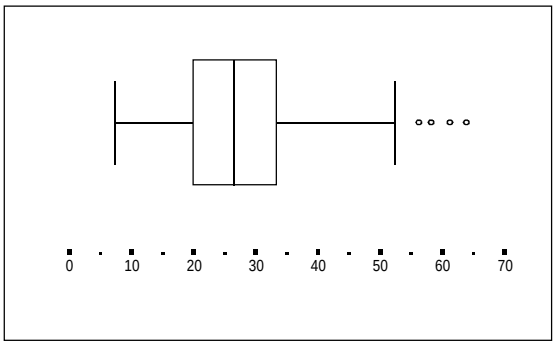
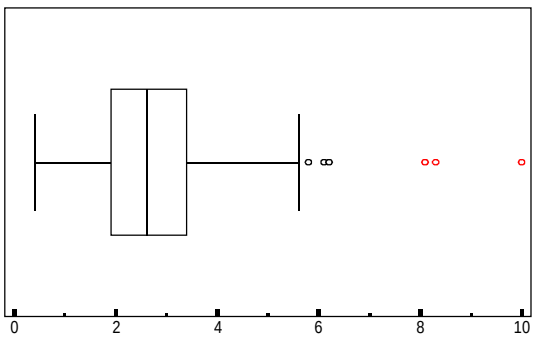
(30.3%) were found healthy at clinical examination, and 106 (69.7%) resulted unhealthy cats with the most clinical manifestation that was lynfoadenomegaly 78 (51.3%) followed by gingivitis 54 (35.5%), ocular infection 29 (19%), upper respiratory tract infection 17 (11.2%) and pale mucous membrane 7 (4.6%).

WBC count was normal in 131 cats (86.2%), RBC was normal in 104 cats (68.4%), concentration of Hb was normal in 125 cats (82.2%), PCV was normal in 91 (60%) cats and PLT count was normal in 133 cats (87.5%). The decreased PCV was the prominent abnormality and it was found in 61 cats (40%), whereas decreased RBC was found in 48 cats (31.6%) and decreased Hb was found in 27 cats (17.8%). Leukopenia was found in 16 cats (10.5%), leucocytosis in 5 cats (3.3%). Neutropenia was found in 2 cats (1.3%) and neutrophilia in 18 cats (11.8%) Lynphopenia was found in 2 cats 1.3%) and linphocytosis in 38 cats (25%). Monocytosis was found in 9 cats (5.9%), eosinophilia was found in 13 cats (8.6%) and eosinopenia in 37 cats (24.3%), basophilia was found in 1 cat (0.7%). Decreased platelets count was found in 10 (6.7%) cats and increased platelets count in 9 cats (5.9%). For normal values see Table 4

Comparison of haematological values between males and females, young and adult cats is reported in Table 2. Comparison of haematological values between pregnant females and non-pregnant females and between healthy and unhealthy cats is reported in Table 3. No significant differences in hematologic parameters were observed between sexes and between different age. Significant difference were observed between pregnant and not-pregnant females and between healthy and unhealthy cats. Compared to not-pregnant females, pregnant females had: significantly lower ($P<0.05$) RBC, Hb and PCV; significantly higher reticulocytes count; quite significantly lower ($P\approx 0.05$) neutrophil count. Compared to healthy cats, unhealthy cats had significantly lower RBC count and quite significant lower Hb and PCV.

Table 1: Data about CBC of feral colony cats of Milan

Parameter	Median Mean ± SD	Min Max	Box-plot
WBC (/µl)	10380 10846 ± 4122	1516 23240	
RBC (x10³/µl)	6460 6522 ± 1069	2100 9190	
Hb (g/dl)	9,2 9.3 ± 1.5	4,7 13,2	
PCV (%)	25.1 25.2 ± 4.1	13.2 38.4	

Parameter	Median Mean ± SD	Min Max	Box-plot
PLT (x103/µl)	378 376 ± 143	90 800	
Neu (%)	64.9 62.5 ± 11.7	26.5 82.9	
Lyn (%)	26.4 28.2 ± 10.9	7.4 63.9	
Mon (%)	2.6 2.8 ± 1.4	0.4 10	

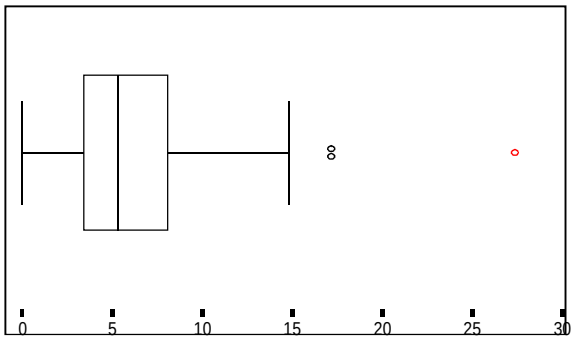
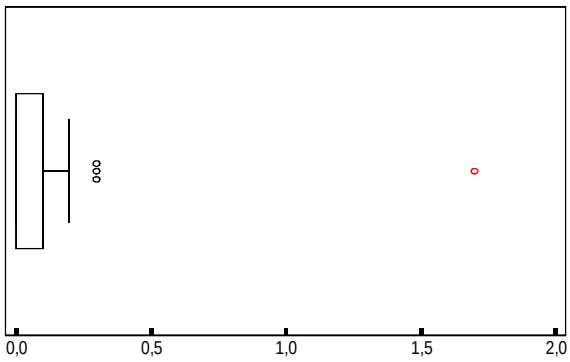
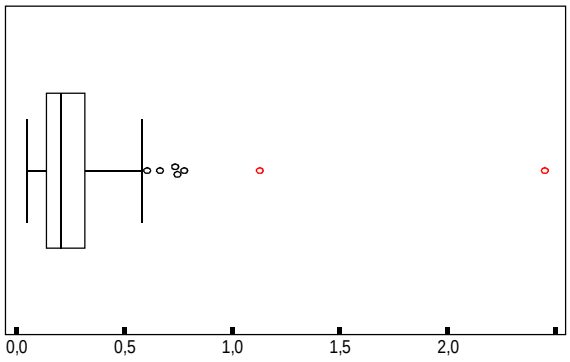
Parameter	Median Mean ± SD	Min Max	Box-plot
Eos (%)	5.3 6.1 ± 3.9	0 27.4	
Bas (%)	0.1 0.1 ± 0.1	0 1.7	
Ret (%)	0.2 0.3 ± 0.2	0.05 2.4	

Table 2: Comparison in haematological values between males and females, young and adult cats

Parameter	Males (41)	Females (106)	p	Adult (81)	Jung (70)	p
	mean±sd	mean±sd		mean±sd	mean±sd	
WBC	10692 ± 4554	10729 ± 3864	0.9607	11140 ± 4008	10399 ± 4176	0.2683
RBC	6576 ± 1118	6509 ± 1055	0.7354	6503 ± 1160	6518 ± 945	0.9313
Hb	9.4 ± 1.6	9.3 ± 1.5	0.7684	9.3 ± 1.6	9.4 ± 1.4	0.6750
Ht	25.2 ± 4.2	25.3 ± 4.1	0.8638	25.2 ± 4.1	25.2 ± 4.0	0.9689
PLT	339± 153	390 ± 139	0.0553	380 ± 123	372 ± 164	0.7397
Neu	60.8 ± 11	63.5 ± 11.6	0.2080	61.9 ± 12.7	63.3 ± 10.5	0.4525
Lynf	30.7 ± 11.8	27.3 ± 10.4	0.0911	28.9 ± 11.8	27.7 ± 9.8	0.5137
Mon	2.6 ± 1.4	2.9 ± 1.5	0.2385	2.7 ± 1.1	2.9 ± 1.7	0.4116
Eos	5.6 ± 3.1	6.1 ± 3.6	0.4529	6.3 ± 4.3	5.8± 3.3	0.3910
Bas	0.09 ± 0.07	0.09 ± 0.2	0.8408	0.10 ± 0.19	0.08 ± 0.07	0.3015
Ret	0.3 ± 0.4	0.3 ± 0.2	0.2506	0.3 ± 0.3	0.3 ± 0.2	0.5876

Table 3: Comparison of haematological values between pregnant and non-pregnant females and between healthy and unhealthy cats

Parameter	Pregnant	Non Pregnant	p	Healthy	Unheathy	p
	mean±sd	mean±sd		mean±sd	mean±sd	
WBC	10189 ± 4236	10846±3795	0.5046	10573±4159	10964±4120	0.5929
RBC	5970 ± 700	6627±1085	0.0133	6841±978	6383±1082	0.0148
Hb	8.5 ± 1.1	9.5±1.5	0.0043	9.7±1.4	9.2±1.5	0.0661
PVC	23.6 ± 3.1	25.7±4.1	0.0471	26.2±3.9	24.8±4.1	0.0516
PLT	355 ± 143	397±138	0.2350	397±151	367±139	0.2454
Neu	67.5 ± 9.1	62.6±11.9	0.0910	61.1±12.7	63.2±11.2	0.3244
Lynf	24.1 ± 6.8	30±11	0.1425	29.4±11.1	27.7±10.8	0.3837
Mon	2.5 ± 1.1	3±1.5	0.1810	2.8±1.5	2.9±1.4	0.6962
Eos	5.5 ± 4.1	6.2±3.5	0.4717	6.4±4.2	5.9±3.8	0.4999
Bas	0.05 ± 0.07	0.10±0.19	0.2545	00.6±00.7	0.10±0.17	0.1514
Ret	0.4 ± 0.3	0.2±0.1	0.0035	0.2±0.1	0.3±0.3	0.4080

Table 4: Hematologic Reference Ranges for Normal Cats

Parameters	**Hematologic Reference Ranges for Normal Cats (61 cats)for ADVIA 120. (Moritz et al. 2004)**
RBC (× 10^3 / μ L)	5920 – 11160
Hemoglobin (g/dL)	8.17 – 15.26
PCV (%)	24.0 – 46.0
Reticulocytes %	0.13 – 0.40
Platelet count (/ μ L)	200,670 – 377,000
Leukocytes (/ μ L)	10,570 – 14,390
Segmented neutrophils	6,100 – 9,480(/ μ L)
Lymphocytes	2,410 – 3,990(/ μ L)
Monocytes	290 – 470(/ μ L)
Eosinophils	200 – 610(/ μ L)
Basophils	Rare 10 – 30(/ μ L)

7.4 **Discussion**

The data collected and shown for the population of feral colony cats in the city of Milan give a picture almost heterogeneous and ranges of the haematological values are very wide: that's because the sample is made of very different subjects many of which showed clinical alterations at physical examination. In literature they say that: age, sex, nutritional status, and husbandry as well as pregnancy, anesthesia, or pharmacologic treatment can affect the measured hematologic values (Rizzi et al. 2010). The effects of most of these factors on hematologic values are not peculiar to the cat, but are common for all domestic species.

The main anomaly found was decrease of PCV even if the fact seemed not to be supported by clinical findings, because only 4,6% of subjects showed pale mucous membranes. PCV under normal values (40%) joined to the low values of RBC (31.6%) and low Hb (17.8%) could be justifiable by the fact that most of the population was made of young cats (46%) and many subjects were infested by parasites (fleas, ancylostoma, trichiurides, coccidia). Besides, we must olso consider that 11% and 67% of the total population was represented respectively

by pregnant females and unhealthy subjects in which PCV, Hb and RBC count resulted significantly lower. Some cats really anaemic in the population could be carrier of systemic of infective pathologies. The leukocytosis found in few subjects (3,3%) could mean a low incidence of infections. The percentage of leukopoenic cats (10,5%) could mean a group of a subjects immunosuppressed or carrier cats of virus (FIV, FeLV).

In our study resulted significant that pregnant cats show a decrease in Hb concentration, low RBC count and reduced PCV in comparison with not pregnant cats while the percentage of reticulocytes in pregnant cats is higher than in not pregnant ones. As support to the above observation it is referred that: queens can exhibit a mild normocytic, normochromic anemia during the last third of pregnancy; this resolves by 1 week post – parturition (Berman, 1974) but here only one study investigated hemogram of the cat during pregnancy (Berman, 1974).

7.5 **References**

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CHAPTER 8

Renal and urinary parameters of feral colony cats in the city of Milan

8. **Renal and urinary parameters of feral colony cats in the city of Milan**

8.1 **Materials and methods** (see also Chapter 2)

8.1.1Urinanalisys

All 61 samples of urine were processed within 3 hours from collection. Specific gravity was measured with a refractometer (Kaneko). Ph and detection of glucose, bilirubine, ketones, albumin, urobilinogen, blood, haemoglobin, nitrates, were measured by dipstick (Combur10 Test UX. Roche). After a low speed centrifugation (1.500-2.000 revolution per minute for 5 minutes) the supernatant was removed and the urine sediment was resuspended in the small remaining volume of urine (0,5 ml) and microscopically observed (40X) to identify and qualify the number of RBCs, WBCs, bacteria, crystals, cells, casts and other. To enhance contrast a sediment stain (kova ® stain TM) was used.

The urinary supernatant was used to determine quantitative estimation of proteinuria by protein/creatinine (UP/UC) ratio. The urine proteins concentration was determined by the pyrogallol red-molybdate complex method, while urinary creatinine concentration was determined after diluition 1:100 by the alkaline picrat method. Analysis were performed using automated analyzer (Cobas Mira Roche) at 37°C.

8.1.2 Serum biochemistry

Shortly after collection the blood in serum tube was centrifuged at 4000 rpm for 10 min, and the serum was removed. For 61 samples serum urea and creatinine were immediately assayed respectively by urea kinetic method and alkaline picrat

method using automated analyzer (Cobas Mira Roche) at 37°C.

8.2 **Statistical analysis**

A Kolmogorow-Smirnov test was used to assess a normal distribution of the parameters (urea, creatinine, UP/UC). The effect of animal sex and age on the various blood and urine parameters were determinated using an unpaired t test. The minimum significance value chosen was $P \leq 0.05$.

8.3 **Results**

Urinanalysis was performed in 61 cats.

Of this 61 cats 45 (73.8%) were intact female, 15 (24,6%) were intact males and 1 (1.6%) was a neutered female. Cats judged to be young were 32 (52.5%), adult were 29 (47.5%) and no one was judged as senior. All cats were European (CE) and living in urban setting.

The 86 % of urine samples was light yellow colour, 6.6% citrine, while 6.6% gold. The apparence was mostly transparent (62%) or slightly turbid (23%) and in 9 cases markedly turbid (14,8%). The average specific gravity for 61 cats was 1056.4 ± 13,4.

From chemical urinanalysis performed by dipstick: no cat was positive for presence of glucose and urobilinogen; in three cases was detected ketones (4.9%) and in 5 cases bilirubin (8,2%); 8 cats showed positivity for blood/haemoglobin/myoglobin, all cats showed positivity for leukocytes and 59 cats (97%) were positive for albumin (traces and +; only 2 cats showed ++). The average Ph for 61 cats was 6,1± 0,5.

The values of serum urea, creatinine, urinary proteinuria (UP/UC) and gravity for 61 feral cats are given in Table 1.

The values of serum urea, creatinine, urinary proteinuria (UP/UC), ph and

gravity for males, females, joung and adult feral cats are shown in Tables 2. Results of microscopical observation of sediment are given in Table 3.

Unpaired t-test showed that: serum urea and urine specific gravity in young cats is significantly higher than in adult cats; Ph in young cats is significantly lower than in adult cats and UP/UC in female cats is significantly lower than in male cats.

Table 1: Values of serum urea, creatinine, urinary proteinuria (UP/UC) and gravity for 61 feral colony cats

Measurement	Mean ± SD	range	Normal range for domestic cats
Urea (mg/dl)	52.8 ±10.2	35-83	15-35[a]
Creatinine (mg/dl)	1.0 ± 0.2	0.6-1.6	0,5-1,8[a]
UP/UC	0.1 ± 0.1	0.0-0.7	< 0.6 [b]
Ph	6.1±0.5	5-7	5-7.5 [a]
USG	1056 ± 13	1025-1070	1035-1060 [c]

[a] From DiBartola 2002
[b] From Iris 2007
[c] From Osborne 1999

Table 2: values of serum urea and creatinine, urinary proteinuria (UP/UC), ph and specific for males, females, joung and adult feral cats.

Measurement	Males (15)		Female (46)		p
	Mean ±SD	range	Mean ± SD	range	
Urea (mg/dl)	55.5 ± 11.7	37-83	52± 9.7	35-72	0.2556
Creatinine (mg/dl)	1.1± 0.2	0.8-1.5	1.0± 0.2	0.6-1.6	0.4178
UP/UC	0.2± 0.2	0.0-0.7	0.1± 0.1	0.0-0.5	0.0305
ph	6.1± 0.5	5.5-7	6.1± 0.5	5-7	0.9306
USG	1054± 17	1025-1070	1057± 12.2	1026-1070	0.4913
Measurement	Young (32)		Adult (29)		p
	Mean ± ds	range	Mean ±SD	range	
Urea (mg/dl)	55.7±8	37-70	49.7 ± 11.6	35-83	0.0226
Creatinine (mg/dl)	1.1±0.2	0.7-1.6	1.0±0.2	0.6-1.6	0.2626
UP/UC	0.1±0.1	0-0.5	0.1±0.1	0-07	0.7779
ph	5.9±0.5	5-7	6.3±0.5	5-7	0.0094
USG	1060±13	1030-1070	1052±13	1025-1070	0.0201

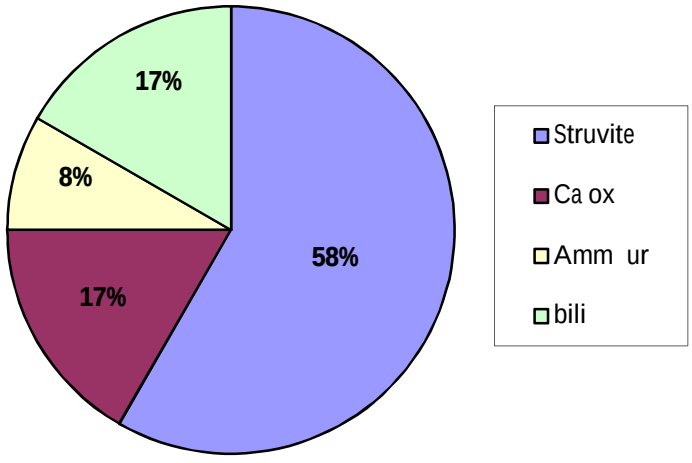
Table 3 : Findings in sediment observation in feral colony cats in the city of Milan

Measurement		Tot (%)	Males	Females	Young	Adult
Crystalls		12 (19.7)	3	9	7	5
	Struvite	7 (11.5)	2	5	3	4
	Calcium oxalate	2 (3.3)	-	2	1	1
	Ammonium urate	1 (1.6)	1	-	1	-
	bilirubine	2 (3.3)	-	2	2	-
Bacteria		5 (8.2)	2	3	3	2
Casts		14 (23)	1	13	8	6
	Hyaline	7 (11.5)	-	7	5	2
	Hyaline-granular	2 (3.3)	-	2	1	1
	Granular	7 (11.5)	1	6	4	3
Epitelial cells		27 (44.3)	7	20	15	12
Transitional cells		23 (37.7)	6	17	11	12
Lipides		38 (62.3)	8	30	17	21
RBC/HPF	>3 [d]	6 (9.8)	1	5	4	2
WBC/HPF	>3 [e]	-	-	-	-	-

[d] Urine sediment performed by cystocenteis normally contains fewer than 3 RBC/HPF (DiBartola, 2002).

[e] WBC are usually in very low number in normal urine (<3/HPF) (DiBartola, 2002).

Graphic 1: Percentage of urinary crystals in Feral Colony cats of Milan



8.4 **Discussion**

Probably, because most of the cats didn't urinated in the cage from the time of capture until the execution of cystocentesis, 90.2% of urine was very concentrated, in most cases with a specific gravity (USG) much higher than 1040. This did not allow us to collect objective data on specific gravity of urine samples of considered cats. Being cat a carnivore animal, urinary pH is acidic and to the normal range is between 6 and 6.5 (G.C. Domenichini, personal communication). Data obtained in our sample are similar to those reported in literature and fall into those normal ranges (Osborne et al., 1999; Reine and Langston, 2005). We found that the urine pH of feral colony cats was significantly different between young and adult cats, with young exhibiting, on average a lower ph (5,9). In the stressed animal the increasing activity of adrenal glands will most likely lead to an increased metabolism, including catabolic conversion of proteins, which, increases sulfidrilic acid production and lower urinary pH come segnalato in un articolo del 2002 (Cottam, et al. 2002). In our case the fact that may have influenced the observed result could be a greater stress associated with capture in young cats.

In 4,9% of cats were detected ketones in the urine in absence of glycosuria, this could be due to a prolonged fast (in case that some cats did not have a chance to eat before the capture). Being urine dipstick a semi-quantitative test for proteinuria more sensitive to albumins than to globulin (and most urine proteins is albumin), very concentrated urine like that of our sample, may cause false positive results; a protein reaction of trace or 1+ may be normal in concentrated urine. In fact, proteinuria found with dipstick is not supported by values of the UP/UC obtained. High percentage of false positive pyuria confirms the uselessness of dipstick for evaluation of this parameter in cat (Reine and Langston, 2005; Iris , 2007).

Subjects with bilirubunuria (8,2%) could be suffering from hepatic disease,

because bilirubinuria is never normal in cats (Reine and Langston, 2005) and it is not found even in highly concentrated urine. (Osborne et al., 1999).

In our sample of feral colony cats in Milan prevalence of bacteriuria was 8.2%; this percentage is higher than that estimated for domestic cats 0.1-1% (Ling, 1995).

In our sample prevalence of crystalluria was 19.7%, in particular, the crystals were composed of struvite (58%), Calcium oxalate (17%), Bilirubine (17%) and Ammonium urate (8%).

We found that the serum urea of feral colony cats was significantly different between young and adult cats, with young ones exhibiting on average a higher urea (55.7 mg/dl) and in general mean value of serum urea in this population of feral colony cats is higher than that reported for domestic cats. This fact, not associated with an increased serum creatinine could be physiologically associated with prolonged fast and with stress caused by capture as reported for European wildcats (Racˇnik, et al. 2004).

We found that the UP/UC of feral colony cats in females was significantly lower than in male cats, but still within the range of normality.

In conclusion, the average values of creatinine and proteinuria observed in feral colony cats in Milan, show a rather healthy population with regard to renal function. This fact, may depend on the young age of the population that does not exceed 10 hears, because Chronic Kidney disease is a condition which affects particularly older cats (Polzin et al., 2002).

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CHAPTER 9

Gastrointestinal helminths parasites in feral colony cats in the city of Milan

9. **Gastrointestinal parasites**

Gastrointestinal parasites are the main cause of morbidity in domestic cats (Khalafalla et al., 2011). From the veterinary point of view, stray cats represent potential reservoir of helminthic parasites to domestic cats, especially in rural areas. Moreover, some gastrointestinal parasites of the cat may represent important zoonoses, and this must be assessed in relation to the fact that cat colonies are often located close to schools, parks, hospitals.

9.1 **Materials and methods** (see also Chapter 2)

During the study a total of 139 faecal samples were collected separately for each individual cat hospitalized in a cage and monitored for 3 days after sterilization. The samples were observed as closely as possible after depositing. Detection of gastrointestinal parasites, eggs, larval stages and oocystis was performed using enrichment by sugar and silver nitrate saturated flotation techniques. After centrifugation for 5 minutes at 1500 rpm the floated material was observed by light microscopy for parasites identification. For detection of *Giardia* a small amount of faeces was mixed with a drop of Lugol's iodine solution on a glass slide, covered with a coverslip and immediately examined at a magnification of 400x. ELISA snap method was used for detection of coproantigens of *Giardia.* The kits are expected to identify antigens in the faeces produced by trophozoites. Most antigen testing has a sensitivity of 100% and a specificity of 96% and identify about 30% more cases of giardiasis in humans compared to zinc sulfate flotation technique (Rosoff et al. 1989).

In a recent study in dogs, an ELISA on faeces was less sensitive and with a lower specificity than the zinc sulfate flotation technique, even if the test is not affected by the expulsion of intermittent cyst (Barr et al.1992)

9.2 **Statistical analysis**

In order to detect the difference in prevalence of parasitic diseases in relation to gender and age, data were tested by Chi-square Test.

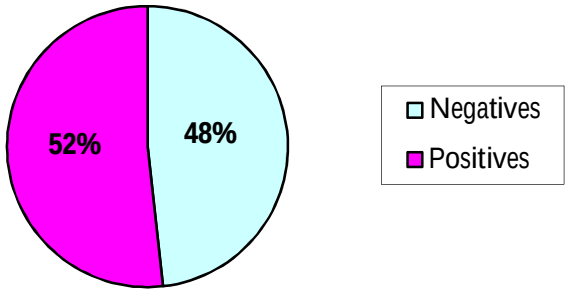
9.3 **Results**

Of 139 cats investigated for gastrointestinal parasites 47 (33.8%) were males, 91 were females (65.5%) and 1 was neutered female (0.7%). Cats judged to be young were 65 (46.8%), adult were 73 (52.5%), no one was judged as senior. Age was not recorded for one cat (0,7%).

All cats were European (CE) and living in urban setting. 3 cats came from colonies located into zone 1 of Milan (2.2%), 5 cats came from colonies located into zone 2 (3.6%), 67 cats came from colonies located into zone 4 (48.2%), 2 cats came from colonies located into zone 5 (1.4%), 15 cats came from colonies located into zone 6 (10.8%), 25 cats came from colonies located into zone 7 (18%), 13 cats came from colonies located into zone 8 (9.3%) and 9 cats came from colonies located into zone 9 (6.5%).

Of 139 faecal samples investigated, 67 (48.2%) were negative, 72 (51.8%) were positive for one or more gastrointestinal parasites. (Graph 1)

Graphic 1: Percentage of feral colony cats positives end negative for research of gastrointestinal parasites in Milan



Among the positive, 61 cats resulted interesting by monospecific infestation (84.7%), 9 (12,5%) by 2 parasitic species and only 2 (2.8%) harbored 3 different parasites at the same time.

Table 1 shows in details the prevalence for the different parasites found. The majority of the co-infestation was rapresented by *ascaris* and *ancylostoma* (3 cases). Statistical analysis showed no significant differences in positivity between males and females ($\varkappa^2$=0.03 p=0.8574) and between joung and adult cats. ($\varkappa^2$=0.73 p=0.3938) (table 2)

Table 1 : Detail of prevalence for gastrointestinal parasites of feral colony cats of Milan

Parasites	Positives n 139	%
Toxocara cati	47	33.8
Ancylostoma	10	7.2
Isospora felis	5	3.6
Dipylidium	4	2.9
Giardia	4	2.9
Aleuristrongylus	4	2.9
Ascaris	2	1.4
Eggs of cestodes	2	1.4
Capillaria	2	1.4
Coccidia	1	0.7
Imenolepis	1	0.7
Tricuris	1	0.7
Isospora rivolta	1	0.7
Lung nematode	1	0.7
Spirometra	1	0.7

Ascaridoidea

Strongyloidea

Coccidia

Cestoda

Trichuroidea

Table 2: prevalence of gastrointestinal parasites in males, females, young and adult cats

	Neg(%)	Pos (%)	Tot
Males	24 (51.1)	23 (48.9)	47
Females	45 (49.5)	46 50.5	91
Young	35 (53.8)	30 (46.2)	65
Adult	34 (46,6)	39 (53.4)	73

9.4 **Discussion**

The survey made, allowed to gain important information on the parasitological state of colony cats in the city of Milan. Copromicroscopical investigations showed an higher positivity for elmintiasis supported by Ascarides (35.2%: *Toxocara cati*, *Ascaris gen*) followed by those supported by the Strongyle (10.8%: *Ancylostoma, Aleuristrongylus, Lung nematode).* These results can be explained by the high environmental resistance that characterizes the infesting elements of these parasites. This study estimates a 51.8% prevalence of intestinal parasites in feral colony cats. This data figure similar to that reported for shelter cats in Japan (43.1%)(Yamamoto et al. 2009); lower than the ones published for stray cats in northern Iran (90% prevalence) (Sharif et al. 2011), mid-Ebro Valley, Spain (89.7%)(Calvete et al. 1998), Rio de Janeiro (90%) (Labarthe et al. 2004), Egypt (91%)(Khalafalla et al. 2011), but the prevalence is higher than the one reported in a metropolitan area of Lisbona, Portugal (23.1%)(Duarte et al. 2010).

However, the comparison of the present study with published surveys indicates that great differences in prevalence were observed for particular parasite species, perhaps due to regional, environmental or climatic variation.

The prevalence of ascaridiosis in Italy was reported with wide range (1-70%) depending on the investigated area (Natale et al 2007). Prevalence of *Toxocara cati*: the dominant gastrointestinal parasite reported in this study (33.8%) is

considerably low in comparison with the prevalence encountered in Greece (67%), Denmark (79%)(Khalafalla 2011), Spain (55%)(Calvete et al. 1998), England (53%)(Nichols et al. 1981), Iran (42.6%)(Zibaei et al. 2007), and in another study developed in Italy (Bologna, firenze, Milano) in 1985 (49.1%)(Poglayen et al. 1985) and higher than prevalence encountered in Veneto (22.4%) (Natale et al. 2007), Japan (21.8%) (Yamamoto et al. 2009), Rio de Janeiro (25.2%)(Labarthe et al. 2004), Egypt (9%)(Khalafalla 2011), Portugal-Lisbona (10.8%).

Prevalence of *Ancylostoma* : the second in prevalence gastrointestinal parasite in this study (7.2%) is similar to that reported for Italy (Bologna, Firenze, Milano) in a previous study (9.5%)(Poglayen et al. 1985) but relatively low in comparison with the prevalence encountered in Veneto (25.0%) (Natale et al. 2007), Rio de Janeiro (74.8%)(Labarthe et al. 2004) and higher than prevalence encountered in Portugal Lisbona(1.4%)(Duarte et al. 2010).

The prevalence of *Isospora felis* (3.6%)in this study is similar to that reported for Lisbona (5,4%)(Duarte et al. 2010). But the prevalence of coccidian in this study (5%) is considerably lower than that reported in Veneto (14.5%) (Natale et al. 2007) and in a previous study made in Italy (12.9%)(Poglayen et al. 1985).

The prevalence of *Dipylidium* (2.9%) in this study lower than that reported in Italy in a previous study (41.4%)(Poglayen et al 1985), and in Spain, mid-Ebro valley (20.7%)(Calvete et al. 1998), and in Iran (49.5%)(Zibaei et al. 2007), in Rio de Janeiro (52.6%) (Labarthe et al. 2004); is higher than that reported for Lisbona (1.4%)(Duarte et al. 2010).

The prevalence of *Giardia* 2.9% is superimposable to that reported for Veneto (2.6%) (Natale et al. 2007) but considerably lower than that reported for metropolitan area of Florence (13.8%) (Papini et al 2007). Since cats shed the organism intermittently; to maximize the possibility of excluding the infection, at least three fresh stool specimens should be examined for a period of three to

five days. That was impossible to be applied in this study so that the prevalence for Giardia may could have been underestimated. The relatively high prevalence rate in this study of cats with gastrointestinal parasites in particular affected by *Toxocara Cati:* an important zoonotic agent suggests that in the city of Milan there is some risk to contract parasitic infection through contact with infected cats and their excretion. Therefore human health education is recommended in developed communities. The TRN program can play an important role for decreasing the number of cats on city ground and the veterinarians should play an important role in increasing the degree of awareness of feline zoonotic parasites .

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